"AND FOR AS MUCH AS IN THE WRITING OF THE SAME, MY PEN IS WORN, MINE HAND WEARY AND NOT STEADFAST, MINE EYES DIMMED WITH OVERMUCH LOOKING ON THE WHITE PAPER AND MY COURAGE NOT SO PRONE AND READY TO LABOUR AS IT HATH BEEN, AND THAT AGE CREEPETH ON ME DAILY AND FEEBLETH ALL MY BODY ..... THEREFORE I HAVE PRACTICED AND LEARNED AT MY GREAT CHARGE AND DISPENSE TO ORDAIN THIS SAID BOOK IN PRINT AFTER THE MANNER AND FORM AS YE MAY HERE SEE."

William Caxton

COMPARATIVE STUDIES OF EVOLUTION IN *GEHYRA* AND *HETERONOTIA* (GEKKONIDAE : REPTILIA)

A thesis submitted for the degree of Doctor of Philosophy

of the Australian National University

CRAIG CHARLES MORITZ

MAY 1984

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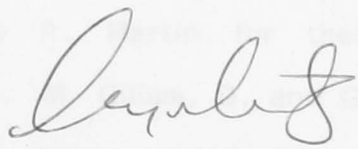
DECLARATION

The research carried out in the course of this investigation and the results presented in this thesis are, except where acknowledged, the original work of the author.



Craig Moritz

May 1984

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ABSTRACT

A comparative study of cytogenetic variation in the geographically widespread gekkonid lizards, Gehyra and Heteronotia, was undertaken to investigate the constraints on chromosome change and the role of karyotypic reorganisation in speciation. In both taxa, emphasis was placed on the application of chromosome banding techniques. The cytogenetic analysis of Gehyra was complemented by electrophoretic, ecological and morphological studies which aimed to evaluate both the population structure of these taxa and the effects of habitat specificity on microgeographic differentiation.

Heteronotia was found to consist of both diploid bisexual populations and parthenogenetic triploid females. The extensive comparative cytogenetic data for these two types of populations provided strong evidence that the considerable clonal diversity of parthenogenetic Heteronotia arose through multiple hybridisation events between genetically distinct bisexual taxa. This study also provided a measure of the extent of evolutionary change occurring within a parthenogenetic lineage. Preliminary studies of genetic variation in three other parthenogenetic gekkonid species are presented and these data are related to current theories on the maintenance of sexual reproduction in vertebrates. The extent of cytogenetic differentiation within these effectively ameiotic parthenogens does not reflect the extensive variability predicted if the requirement for correct meiotic segregation acts as a major barrier to the establishment of chromosome rearrangements.

The chromosome banding analysis of Gehyra demonstrated the presence of polytypic Robertsonian and tandem fusions. Additionally, numerous polymorphisms for other forms of chromosome change were

identified. In G. purpurascens, a highly variable W chromosome was shown to have become differentiated by multiple inversions. The evolution of highly variable W chromosomes in this species and also in Heteronotia is related to current theories on the evolution of sex chromosomes. It is proposed that these chromosomes may be subject to elevated mutation rates.

Ecological studies of Gehyra revealed that populations with broad ecological requirements are capable of the rapid recolonisation of vacant habitats and may also effect gene flow through nesting excursions by gravid females. The level of microgeographic differentiation as measured by both electrophoretic and cytogenetic markers is substantially lower in such taxa than it is in rock restricted forms of Gehyra.

Analyses of morphological variation in Gehyra indicate that the external appearance of these geckos in principally determined by their ecological requirements rather than their phylogenetic affinities. Congeneric competition appears to be critical in determining patterns of habitat use and thus morphological variation.

The available data for Gehyra do not appear consistent with the regular formation of the extremely small demes necessary if single underdominant chromosome changes are to act as causal mechanisms in speciation. Alternative modes of chromosomal speciation that do not require such small demes are briefly discussed.

CHAPTER 1

INTRODUCTION

1.1 Models of Speciation

There has been a dramatic resurgence of interest in the study of speciation during the past fifteen years. One reason for this attention relates to the controversy over the nature of gaps in the fossil record which has stimulated renewed interest in mechanisms that allow for rapid evolutionary change (Grant 1982). One school of paleontologists has recently emphasised the concentration of morphological change within the fossil record into short geological time spans and suggested that the speciation mechanisms thought to be responsible for macroevolutionary changes are qualitatively different to microevolutionary processes (reviewed by Levinton 1983, Stanley 1979, Grant 1982, White 1979, 1982). This view has been strongly contested by several neo-Darwinian population geneticists (Charlesworth et al. 1981, Mayr 1982, Grant 1982, Wright 1982) who argue that a rapid change in a geological time frame in fact allows sufficient time for microevolutionary processes to result in the observed changes. While, as Carson (1982) pointed out, polemics have frequently been substituted for reasoned scientific exchange in these arguments, the punctuated equilibria controversy has served to focus attention on the issue of rapid evolutionary change and speciation in particular.

The second factor which has caused renewed interest in the study of speciation concerns to the significance of gene flow. The exchange of genetic material between populations has frequently been regarded as a strongly cohesive force in evolution (Dobzhansky 1970; Mayr 1970; Grant 1981) which led to the supposition that geographic

isolation was an essential precondition for the establishment of reproductive isolation. Recent reviews of speciation theory have questioned this conclusion and emphasised the plurality of speciation mechanisms (Bush 1975; Endler 1977; White 1978a).

The various models of speciation are generally categorised according to the geographic context in which reproductive isolation was assumed to have been attained (reviewed in Bush 1975; Endler 1977; White 1978a; Mayr 1982). Allopatric and parapatric models emphasise the importance of geographic isolation and divergent selection pressures causing adaptation to different local environments. Parapatric or clinal speciation models suggest that strong disruptive selection can cause reproductive isolation where restricted gene flow is present. Sympatric speciation theories consider the effects of disruptive selection on the separation of gene pools which are potentially capable of extensive genetic exchange.

This preoccupation with the geographical context of speciation has frequently resulted in sterile debates over the extent of gene flow between specific taxa at the time when reproductive isolation was achieved. As with other critical parameters in the study of speciation, this factor has to inferred from the characteristics (in this case the distribution pattern) of the extant populations which, in effect, nullifies the predictive power of these geographically based speciation models. Divergent preconceptions have produced a literature replete with examples of opposing, but equally plausible, geographic interpretations of specific cases. Three examples should clearly reveal the contentious nature of zoogeographic reconstructions.

(i) White (1978a) considered that the most parsimonious explanation for parapatrically distributed taxa was that of in situ divergence unless it could be proven that the taxa in question has been physically separated at some time. In contrast, Mayr (1982) claims that all parapatric distributions are the result of secondary contact between taxa that had differentiated in allopatry. As Endler (1977) has emphasised, it is rarely possible to objectively distinguish these alternatives.

(ii) Virtually all of the cases proposed by White (1978a) to exemplify stasipatric or sympatric speciation can be reinterpreted as examples of allopatric speciation (Futuyma and Mayer 1980; Key 1981). In fact, Mayr (1982) suggests that these cases represent clear illustrations of allopatric speciation on mainlands - a process that White (1978a) considers rare.

(iii) White (1978a) proposed that extreme levels of endemism in some taxa on isolated islands offered strong evidence for sympatric speciation yet, on equally plausible grounds, Mayr (1970, 1982) regards these cases as examples of multiple invasions or peripatric speciation.

On such controversial criteria, a geographically based classification of speciation mechanisms appears to be explanatory rather than predictive (Templeton 1981). While the relationship between gene flow and speciation is undoubtedly an important question, it is unlikely that the use of zoogeographic reconstructions

as the principal evidence will be illuminating. It therefore seems preferable to concentrate on the genetic mechanisms (in the broad sense) of speciation. It is the mechanisms of genomic change in a speciation event that will determine the impact of gene flow on the establishment of reproductive isolation and not vice versa.

This thesis is primarily concerned with the constraints that act upon the establishment of chromosomal mutations and the role that these mutations may play in the speciation process. However, prior to describing the aims and approaches adopted in the study, it is first necessary to outline the theoretical framework applicable to speciation and more specifically to chromosomal rearrangements. This review consists of a classification of the principal mechanisms of speciation, a brief discussion of non-chromosomal mechanisms and a more detailed review of the current models of chromosomal speciation.

The experimental approach to speciation, defined and applied by Templeton (1980a,b 1981, 1982a) represents an attempt to make the study of speciation more predictive. White (1978a, 1982) recognised the need for classifying the principal mechanisms of speciation but refrained on the grounds that our rapidly accumulating knowledge of genomic change is too incomplete to allow precise definition of the causal factors involved in cladogenesis. In this he was undoubedly correct. For example, Raff and Kaufmann (1983) list 20 categories of genomic change and for most of these, the ensuing genetic and phenotypic consequences are obscure. Nevertheless, an approximate classification based on genetic mechanisms, no matter how archaic it may become within the next decade, is preferable to one based purely on zoogeographic inferences.

The scheme presented in Table 1.1 concentrates on the major mechanisms of speciation that are thought to be relevant to animals. Thus hybridisation and polyploidy which are important mechanisms in plant speciation (Grant 1981) but are considered rare in bisexual animals (White 1978a), are excluded. Also, cytoplasmic factors such as viruses and mycoplasmas, which appear to cause asymmetrical hybrid sterility in some Drosophila (Dobzhansky 1970) and Culex species (Powell 1982), are not considered since the generality of these mechanism is not clear.

It should be emphasised that the critical genomic alterations underlying the formation of species through pleiotropic affects or changes in regulatory genes have never been precisely defined or identified. Highly or moderately repeated DNA, transposable elements in particular, and molecular drive have all been implicated (Schope 1981; Flavell 1982; Dover et al. 1982; Peacock et al. 1982) however direct evidence for their role in cladogenesis in lacking. In particular, this applies to the highly repetitive fraction of the genome (Miklos et al. 1980; John 1981; White 1982).

The three basic categories in Table 1.1; pleiotropic divergence, regulatory gene changes and structural rearrangements of chromosomes, are closely interrelated. This is most evident when the consequences of evolution in small populations are considered. Under these conditions speciation has been suggested to result from changes in regulatory processes (Wilson et al. 1977b), establishment of negatively heterotic chromosome rearrangements (White 1978a), as a pleiotropic consequence of disruptive selection due to changes in the genetic background (Mayr 1970, 1982; Carson 1982; Templeton 1980a), changes in mate recognition systems (Kaneshiro 1980), or a

Table 1.1 A tentative mechanistic classification of speciation mechanisms. In many cases, and the pleiotropic effects in particular, the postulated genetic mechanisms may only represent correlates of more fundamental genomic chances. The concept employed in this classification is attributable to Templeton (1981) although the scheme presented here is distinct in several respects. This classification excludes hybrid maintenance and recombinational speciation and includes an additional class of chromosomal speciation (3-i). The approximate synonymies between Templeton's classification and this one are; divergence = 1a, genetic transilience = the second category of 1b, chromosomal transilience = 3 ii.

CAUSAL FACTOR	PRINCIPLE GENETIC MECHANISMS	POSSIBLE PHENOTYPIC CONSEQUENCES	RELATIVE RATE
1. PLEIOTROPIC EFFECTS			
a) Large populations	Changes in polygenic characters and structural loci. Differential co-adaptation	Morphological change Ecological shifts?	SLOW
- sexual selection		Change in mate recognition	RAPID
b) small populations	Reduced variability of polygenic systems leading to loss of coadaption and thus disruptive selection OR changes in major gene systems with little effect on polygenic systems	morphological change mate recognition change ecological shifts?	RAPID
2. REGULATORY GENE CHANGES	Changes in developmental sequences or gene regulation in small populations (drift) or through alterations of linkage groups via chromosomal rearrangements	morphological change? reduced F_1 viability	RAPID
3. STRUCTURAL REARRANGEMENT OF CHROMOSOMES	(i) Changes in pattern of recombination leading to differential coadaptation. Disruption of coadaption by recombination in F_1	Reduced viability of F_2 or backcross	GRADUAL
	(ii) Direct effects of structural heterozygosity on meiotic segregation patterns - aneuploidy and segmental duplications and deficiencies	Reduced fecundity of F_1 +/or reduced viability of F_2 or backcross	RAPID

combination of these factors. The categories listed in Table 1.1 are briefly considered below.

(i) PLEIOTROPIC EFFECTS

This class of models suggest that both pre- and post mating reproductive isolation develops as a byproduct of other genetic changes occurring in either large populations or small isolates. The latter may be a normal component of population structure (i.e. highly subdivided populations) or may arise through founder effects or occasional population bottlenecks.

The classical dumbell model of allopatric speciation (Mayr 1970) suggests that two (or more) large isolated populations will gradually diverge by way of differential adaptation to their respective environments. The underlying genetic changes are considered to involve polygenic or structural genes rather than elements involved in gene regulation (Bush 1975; Hedrick and McDonald 1980; Templeton 1982a) and will in general, lead to appreciable genetic distances between species if the process is gradual (Ayala 1982, Templeton 1980b).

While Mayr (1970) regarded large populations as relatively inert in a cladogenetic sense, reproductive isolation can, in theory, be rapidly acquired where strong sexual selection acts on complex mate recognition systems (Lande 1982, Templeton 1982a). Lande (1982) proposed that this mechanism does not require geographic isolation of the incipient species. It has also been suggested that premating isolation can evolve in sympatry where closely linked mutations for host recognition, host selection and strong assortative mating occur (Maynard Smith 1966; Bush 1975). These latter models were

criticised on theoretical and empirical grounds by Futuyma and Mayer (1980).

Considerable emphasis has been placed on the cladogenic potential of genetic changes in small populations. These models had their beginning in Wright's shifting balance theory of evolution which effectively coupled random genetic perturbations, resulting from small population size and inbreeding, with selection operating on epistatic genetic systems (reviewed by Wright 1982; Templeton 1982a). From the enormous literature on this subject there appear to be two rather distinct mechanisms for generating evolutionary changes.

Mayr (1970, 1982) and Carson (1982) have emphasised the induction of a "genetic revolution" accompanying a rapid reduction in population size. Their argument, considerably simplified, is as follows. The colonisation of vacant habitat by one or very few individuals will lead to the disorganisation of a polygenically balanced gene pool by stochastic forces over a small number of generations. In the second anagenetic phase, the genome gradually aquires a new coadaptated system as a result of stabilising selection. Carson (1982) stressed the necessity of a preceeding population flush in which selection was supposedly relaxed, thereby allowing increased variation amongst polygenic systems otherwise constrained by the requirement of coadaptation. The principle phenotypic consequences suggested by these authors include morphological divergence and changes in mate recognition systems. Experimental evidence for the latter has been provided by Powell (1978).

The genetic revolution suggested by Mayr and Carson involves a dramatic reduction in heterozygosity and leads to the prediction of substantial genetic distance accompanying speciation (Templeton 1980b but see section 1.4). Templeton (1980a) suggested an alternative

scheme, that he called genetic transilience, in which less drastic and more temporally restricted founder events operate. These are proposed to result in the rapid divergence of major genes controlling aspects of development, physiology and behaviour, but would have minimal impact on polygenic systems or overall levels of heterozygosity. Thus a founder event of this sort, while inducing rapid change, allows the retention of sufficient genetic variation to provide a basis for a response to selection. The ecological and genetic conditions required for a genetic transilience are considerably more restrictive than those envisaged by Mayr and Carson but Templeton favours this hypothesis as an explanation of the Hawaiian Drosophila radiations.

(ii) CHANGES IN REGULATORY GENES

The second general category, referred to in Table 1.1, regulatory gene changes, has frequently been invoked as a general explanation for both rapid phenotypic changes accompanying cladogenesis (Stanley 1979; Raff and Kauffman 1983) and more gradual adaptive change (MacIntyre 1982; McDonald 1983). This proposal has been supported by two kinds of arguments.

Firstly, there is now considerable evidence that changes in those structural genes assayed by electrophoresis, are not causally related to rapid adaptive change or cladogenesis (Ayala 1982; Nevo and Cleve 1978; Wilson et al. 1977a). It has therefore been argued, by elimination, that regulatory gene changes are more important as a causal mechanism for such change (Wilson et al. 1977b).

Secondly, in a series of comparative studies of rates of evolution of mammals, birds and frogs, A.C. Wilson and his collegues have demonstrated a positive correlation between the rate of loss of

hybridisation potential and the rate of morphological evolution and speciation (Wilson 1975; Wilson et al. 1974a; 1977b). This is interpreted as strong evidence for the primary role of changes in gene regulation since the data indicate the rapid development of developmental barriers to hybridisation in the virtual absence of structural gene changes.

The required changes in gene regulation could be mediated by two distinct processes - mutations at controlling loci or gene rearrangement. The former mechanism is often implicated in discussions of rapid cladogenesis in small populations (Templeton 1980a; and Carson 1982). The difficulty here is that the current knowledge of regulatory changes is highly speculative and fragmentary although, it is generally accepted that both the temporal and spatial regulation of gene activity in eukaryotes is the result of a complex hierarchical procedure (Davidson and Britton 1979; MacIntyre 1982; Raff and Kaufmann 1983). Fundamental changes in development and physiology would therefore require mutations at the lower level of this hierarchy. While such basic mutations have been studied in the laboratory (e.g. homeotic mutants in Drosophila - reviewed by Raff and Kaufmann, 1983) regulatory gene variation in natural populations appears to be restricted to mutations causing minor effects such as enzyme concentration (MacIntyre 1982; McDonald 1983). Thus McDonald (loc. cit.) suggested that the changes in gene regulation required for fundamental phenotypic changes cannot be accounted for by mutations segregating in natural populations and must therefore be created de novo.

One frequently proposed mechanism for achieving this end is gene rearrangement resulting in altered spatial relationships between

major regulatory genes and the loci with which they interact. Several authors, and most prominantly Wilson and his collegues, have therefore suggested that the most profound effect of structural chromosome rearrangements is to alter the linkage relationships of regulatory genes (Wilson et al. 1974b, 1975; Bush et al. 1977; Bush 1981 and see also Bickham and Baker 1979). Through a comparison of rates of gross structural chromosome change in mammals and other vertebrates Wilson et al. (1974b) and Bush et al. (1977) claimed that a causal relationship existed between the establishment of chromosome rearrangements and the rapid changes of regulatory genes evidenced by phenotypic change and F_1 hybrid inviability.

This conclusion has been contested on two grounds. Firstly, the results of their extremely broad comparisons (between divergent taxonomic levels) are not confirmed by more detailed analyses at lower taxonomic levels. There are several examples of radical chromosomal repatterning separating taxa without fundamental phenotypic consequences (Liming et al. 1980; Baker and Bickham 1980; Christidis 1983). In addition, the predicted correlation between chromosome change and morphological divergence is either absent (Schwenck et al. 1982; Larson et al. in press), or reversed (Gold 1980), when lower taxonomic levels are compared.

The second major criticism concerns the proposition that certain mammalian taxa have a higher rate of chromosome evolution than lower vertebrates. It has been noted that both Wilson et al. (1975) and Bush et al. (1977) severely underestimated the extent of karyotypic variability in bats (Bickham and Baker 1979) and reptiles (King 1981) and if the cytogenetic data employed for the rapidly evolving groups (primates, horse and rodents) were more comprehensive than those

for other taxa, this could at least partly account for the observed disparity. Another inherent assumption of the analysis was that rates of karyotypic change are approximately constant within a lineage, whereas Bickham and Baker (1979) have proposed that chromosome change may be concentrated in a short period of time early in the initial radiation of a family or order. Averaging the extent of chromosome variation by the age of a lineage would then lead to ancient taxa having apparently slower rates of evolution simply because they are older. A dramatic example of variation in the rates of chromosomal evolution is provided by Australian members of the genus Rattus (Baverstock et al. 1983a). The dependance of the calculated rates of evolution on time intervals (Gingerich 1983) is strongly evident in the data of Bush et al. (1977) and their conclusions therefore require verification using taxa that have differentiated over similar periods of time. That the relationship between chromosome change and morphological divergence is not supported by comparison at lower taxonomic levels (see above) suggests that the correlation observed by Wilson et al. (1975) and Bush et al. (1977) is an artifact.

On the basis of the available data it must be concluded that if gene rearrangements are important in causing fundamental changes in gene regulation they are the result of a more subtle process than gross structural rearrangements of chromosomes (Raff and Kaufman 1983). A plausible alternative that is currently in vogue is that transposable elements may mediate regulatory changes (Peacock et al. 1982; Raff and Kaufman 1983). This proposition is at least supported by some experimental data (McClinlock 1978; Fedoroff 1983) and advances in this area of molecular biology should allow this hypothesis to be critically tested in the near future.

1.2 Models of Chromosomal Speciation

In addition to the speculative proposal that gross structural chromosome changes can effect gene regulation there exists two other ways in which chromosome changes can lead to post-mating isolation and thus speciation. The first of these, which is considered as the primary effect of chromosome rearrangements by many authors (e.g. Grant 1956; Shaw 1981; Hayman 1981), concerns the effect on recombination. If structural rearrangements of chromosomes result in changes in the spatial distribution of chiasmata or their frequency, then populations that have fixed differences for the rearrangements may gradually acquire differentially coadapted segments. In hybrids between these populations, recombination between these segments could lead to the disruption of the internally balanced chromosomes and thereby reduce the viability of the subsequent generation (Shaw 1981, Coates and Shaw in press). It should be noted that, if there are no other meiotic consequences of the rearrangements, their initial fixation in a deme would be facilitated by, but does not require, small deme size.

This "recombinational breakdown" has been demonstrated as a major component of the reduction in F_2 and backcross viability between taxa of the grasshopper Caledia captiva which differ by a series of pericentric rearrangements (Shaw and Coates 1983; Coates and Shaw in press). The relevance of this mechanism to other types of structural rearrangements (i.e. interchromosomal rearrangements) is not clear. John and Freeman (1975) suggested that Robertsonian fusions will generally lead to a reduction in chiasma unless, of course, recombination was previously restricted to the distal regions of the chromosome. In the grasshopper Podisma pedestris, Hewitt

and John (1972) found that an X-autosome fusion resulted in the redistribution of chiasmata with an associated reduction in chiasma frequency however there has been no analysis of the F_2 or backcross progeny of hybrids between these races (Barton 1980; Barton and Hewitt 1981a). A spontaneous fusion heterozygote was studied by Southern (1967) who found no reduction in chiasma frequency in the heterozygote relative to that in homozygous individuals.

Linkage tests in Mus musculus heterozygous for Robertsonian fusions have produced particularly interesting results (Cattenach 1978) when Mus musculus acrocentrics were combined with their metacentric homologues derived from Mus poschiavinus, only three of the eleven combinations tested showed crossover suppression in the proximal region. Furthermore, in two of the three cases the effect was asymmetric; only one arm of the metacentric being affected. (Cattenach loc. cit.) also tested Robertsonian fusions derived from Mus musculus and could find no evidence for crossover suppression in these cases. Faced with this variation, Cattenach (loc. cit.) concluded that the effect on recombination was not on effect of the centric fusions per se, but instead could be due to minor structural rearrangements in the proximal regions of some metacentrics or to genic effects. Note that if genic effects were the underlying cause of the effects on recombination, then they are not the same as those postulated to effect rates of non-disjunction in Mus (see below). Clearly, further studies of the effects of chromosome rearrangement on recombination and the effects of hybridity are required before the generality of recombinational breakdown can be assessed.

The second postulated effect of gross structural chromosome changes concerns their transmission in meiosis. Many authors, and

White (1973, 1978a) in particular, have advocated the view that some types of rearrangements lead to appreciable levels of aneuploidy or segmental duplications and deficiencies when present in a heterozygous condition. This would lead to decreased fitness in F_1 hybrids and may therefore constitute an effective post-mating barrier to gene flow. Before the evidence for this meiotic barrier is reviewed (section 1.3), the theoretical conditions for the establishment and spread of chromosome mutations that behave in this manner will be considered.

The conditions for the establishment of chromosomal rearrangements that cause a substantial reduction in the fitness of heterozygotes have been studied by Wright (1941), Bengtsson and Bodmer (1976a); Lande (1979); and Hedrick (1981). The conclusions of these authors are summarized below. Fixation of the rearrangement within a deme by drift alone is improbable where the selective deficits of heterozygotes (S) is greater than 0.1 (in general, negligible probability of fixation for $S < 1/N$). However, rate of the fixation for those that do survive is higher than for a neutral character. The probability of fixation by drift is increased if (i) the level of inbreeding is also high (ii) meiotic drive favours the new rearrangement, (iii) the mutation rate is increased and (iv) the new homozygote has a selective advantage over the original homozygote. Strong meiotic drive can also lead to the rapid fixation of rearrangements in the absence of drift, inbreeding or adaptive effects of the new homozygote.

Once established within a deme, there are two ways in which the rearrangement may spread. White (1968, 1978a) envisaged a process called stasipatry in which the rearrangement arises in a deme within

the distribution of the parental taxon and then forms a narrow parapatric zone of hybridisation (a tension zone, see Key 1981). By virtue of the adaptive superiority of the homozygotes, or through strong meiotic drive, the tension zone gradually moves into the territory of the ancestral karyomorph. Key (1981) suggests that as other genetic differences accumulate within this tension zone, the post-mating barrier to gene flow becomes more effective.

This process has been modelled and simulated by Barton (1979) and Spirito et al. (1983) however their conclusions are based on deterministic models that take no account of drift. These authors found that unlinked neutral genes will readily pass through the hybrid zone where $S < 0.3$ and even greater levels of negative heterosis are required before introgression of adaptive genes is prevented. White (1978a) suggested that a selective deficit of 5 to 10% in heterozygotes is sufficient to initiate divergence but it is clear that much greater values of S are required for a tension zone to operate in the manner suggested by White and Key. If strong assortative mating also operates then a lower selective deficit may be effective (Bush 1981; Barton and Hewitt 1981b). However, with the possible exception of Spalax (Nevo and Heth 1976; Heth and Nevo 1981; Nevo et al. 1975) premating isolation has not been demonstrated in chromosomal hybrid zones (Barton and Hewitt 1981b; White 1978a). Further, several authors have argued that the evolution of premating isolation within a hybrid zone (reinforcement) is highly improbable (Templeton 1981; Lande 1982; Paterson 1978).

The second mechanism for the spread of a new chromosome rearrangement concerns the impact of colonisation. Lande (1979) suggests that a rearrangement may rapidly spread in the homozygous

condition if deme sizes are small (tens to hundreds), extinctions and colonisations are frequent, and the colonists of a particular area are derived from a single deme. This process will be facilitated by meiotic drive or selection favouring the new homozygote. Key (1981) considered this model to be ecologically unrealistic; however once several demes containing the new rearrangement at high frequency can act as source populations, Lande's (1979) model becomes more plausible.

A variant on this theme is provided by King (1981, primary chromosomal allopatry) and White (1982, invasive chromosomal speciation). These latter models suggest that the rearrangement occurs on the periphery of the progenitor's distribution and expansion of the range of the new karyotype is promoted by colonisation of vacant habitat. As discussed by King (1981), the initial stage of this mechanism is presumed to be associated with a founder effect.

In summary, to act as effective post-mating isolating barriers, chromosomal rearrangements must incur appreciable negative heterosis ($S > 0.3$). Genetic drift alone cannot, at any realistic level of probability, lead to the fixation of these rearrangements unless combined with strong inbreeding, meiotic drive or adaptive superiority of the novel homozygote. The subsequent spread of the rearrangement then requires either of the latter two effects (for stasipatry) or colonisation of individuals homozygous for the rearrangement. In the case of Lande's (1979) model, deme size must remain small and extinctions must be frequent.

Evidence favouring either meiotic drive in the appropriate direction or the adaptive superiority of novel homozygotes has proved elusive. Of the three possible examples of meiotic drive effecting

chromosome rearrangements, two operate in the wrong direction (Vandiemenella; Mrongovius 1979, Mus; Gropp and Winking 1981) and the third occurs in a system where Robertsonian fusions have absolutely no effect on F_1 fitness (Sheep; Bunch and Foote 1977; Valdez et al. 1978). The significance of this process in chromosomal speciation therefore requires clarification (Shaw 1981).

The direct adaptive effects of polytypic chromosome variants, although widely accepted (White 1978a; Mayr 1970, 1982, Bickham and Baker 1979), have proved difficult to substantiate. As White (1978b) comments, adaptive linkage effects may be more relevant to selectively balanced polymorphisms (reviewed in White 1973).

The adaptive role of chromosome change can be envisaged to act in terms of two processes. Firstly changes in linkage relationships may favour the accumulation of differentially coadapted complexes (Grant 1956; Shaw 1981). However, despite the evidence that rearrangements may lead to a modification of recombination patterns (see above), obvious phenotypic manifestations of this process have rarely been detected (Shaw 1981). In this regard, it is pertinent that Key (1981) found that most tension zones are not associated with obvious ecotones. Two possible cases in which this process may have operated are the association of Robertsonian fusion variants with exposure to wave action in Nucella lapillus (Bantock and Cockayne 1975) and the correlation between aridity and chromosome number in Spalax (Nevo et al. 1979; 1982a,b). Patton and Sherwood (1983) view the frequently cited examples of environmental correlates of chromosome change with scepticism since the role of the chromosome rearrangements per se has not been demonstrated. It must be emphasised that the construction of differentially coadapted regions of

the genome is gradual and would not result in the rapidly aquired adaptive superiority required to facilitate the establishment and subsequent spread (by stasipatry) of negatively heterotic rearrangements.

The second postulated link between chromosomal repatterning and speciation involves a direct effect on gene regulation. Major effects on gene regulation, such as are required for large scale adaptive shifts and speciation, do not appear to be correlated with chromosome change (see above). However, it is possible that minor adaptations, involving regulatory genes higher in the hierarchy, may be induced by chromosome change (Bush 1981). At present there are no examples of this process in eukaryotes, but they have hardly been looked for. In this respect it would be interesting examine individuals with spontaneous chromosomal mutations (e.g. in laboratory stocks of Mus) for the predicted minor changes in gene regulation which may alter the concentration or expression of metabolic enzymes.

Small population size is a prequisite for the establishment of negatively heterotic chromosome rearrangements. As a consequence there have been numerous reports of a correlation between deme size and rates of chromosome evolution (Arnason 1972; Wilson et al. 1975, 1977b; Bush et al. 1977; Bush 1981; King 1981; White 1978a,b). The data of Wilson et al. (1975) and Bush et al. (1977) have already been discussed with respect to regulatory gene evolution. However, despite the methodological difficulties in their studies, it cannot be denied that certain groups of mammals, i.e. rodents, primates and horses, have experienced or are still undergoing rapid chromosomal change. The critical question relates to the deme sizes in these

organisms and if they are sufficiently small to allow the establishment of a rearrangement that would lead to post-mating isolation?

For small mammals the answer appears to be no (Moritz 1982, Daly 1981; Schwartz and Armitage 1980; McCracken and Bradbury 1977; Patton and Sherwood 1983). For example, detailed studies of Spalax and Thomomys, both regarded by White (1978a) as clear cases of chromosomal speciation, have revealed population structures that are not compatible with the requirements stated above (Nevo et al. 1982c; Patton and Feder 1981). Therefore if chromosome rearrangements that significantly depress the fitness of heterozygotes are to become established, they must do so in rare isolated populations of these species.

Members of the subgenus Mus have been regarded as the paradigm of both small inbred demes and stasipatric speciation (Wilson et al. 1975, Bush et al. 1977, White 1978b). This presumption is based on the observed cohesive social groups and genic heterogeneity between some commensal populations (Anderson 1970). However this pattern of restricted gene flow is not general for all commensal populations and, furthermore, natural populations appear to be fundamentally different (Bronson 1979; Berry 1981; Sage 1981; Baker 1981). The commensal nature of Mus in the Italian Alpine regions (Sage 1981; Capanna 1982) may have facilitated the establishment of chromosome rearrangements but whether the effective population size is low enough to allow strongly underdominant mutations to become established requires verification.

The sweeping generalisations of Wilson et al. (1975) and Bush et al. (1977) do not appear to be supported when lower taxonomic levels are examined. Thus there are no evident correlates between

population size and cases of dramatic karyotypic organisation in bats (Baker and Bickham 1980) or lemmings (Gileva 1983). Similarly it seems unlikely that population structure alone can account for the existence of extensive Robertsonian fusion variation in some sections of the subgenus Mus (Sage 1981, Capanna 1982; Adolph and Klein 1983; Brooker 1982) while a further six species of the same subgenus cannot even be distinguished by G-banding analysis (Hsu et al. 1978). From this discussion it is evident that if small deme size is to be considered as an explanation of rapid chromosome change it must be demonstrated in the taxa in question.

1.3 Restrictions on Chromosome Change

The constraints on the establishment of structural chromosome rearrangements are of four types; (1) mutation rate, (2) mitotic segregation, (3) gene disruption and (4) meiotic segregation (White 1973). This discussion will exclude genomic rearrangements such as polyploidy and changes in the position and magnitude of segments of highly or moderately repetitive DNA and ribosomal genes.

(i) Mutation

It is a truism that all structural rearrangements of chromosomes are derived from mutation events, but how significant is this process as a controlling factor in chromosome evolution? The spontaneous mutation rate for chromosome rearrangements is extremely difficult to assess. White (1973) suggested a figure of 10^{-6} spontaneous breaks per locus per generation and the frequency of reciprocal translocation mutations is variously estimated at 10^{-3} to 10^{-4} per generation in human and grasshoppers (White 1973; Lande 1979).

It has recently become apparent that the chromosomal mutation rate may vary in time and space within a species. With the rediscovery of transposable elements several authors have suggested that they may at least partly explain some examples of rapid chromosomal change (Baker and Bickham 1980; Bush 1981; Patton and Sherwood 1983; Larson et al. in press). Indirect evidence for this process has been obtained from cytological studies of hybrids between incipient species (Shaw et al. 1983) or between allopatric conspecific populations (Peters 1982) which demonstrated increased chromosomal mutation rates. The evolutionary role of transposons must be considered with caution since our knowledge of the control and frequency of transposition under natural conditions is fragmentary. Although there is strong case for their mobilisation by genomic stress (McClintock 1978; Shaw et al. 1983) there is no evidence that environmental stress will cause their expression as McDonald (1983) has envisaged. The relevance of transposable elements to chromosomal change in the absence of genomic stress (e.g. hybridisation) remains to be demonstrated.

(ii) Mitotic Stability

Once the mutations have been generated they will not persist if they show mitotic instability. White (1973) lists several forms of chromosome mutation that would not be expected to survive mitotic division. These include breakage without reunion, sister strand reunion, and under some circumstances, the formation of dicentric chromosomes.

(iii) Gene Disruption

If the breakpoint of a chromosomal mutation occurs within a structural gene or a controlling element then the inevitable disruption of its function may result in lethality (Lefevre, 1973). Direct effects of this sort appear to be rare in plants (Grant 1981) but are generally deleterious in animals (White 1973) and may therefore be an important factor in the elimination of chromosome rearrangments (White 1975).

An instructive series of experiments in Drosophila has suggested that gene disruption may be restricted to those genes immediately adjacent to euchromatic breakpoints. Spradling and Rubin (1983) found that the artificial transposition of the xanthine dehydrogenase gene, together with short flanking sequences, into other sites in the genome had minimal effects on its expression and regulation. They therefore concluded that large chromosomal domains were not required for the correct regulation of this gene.

A distinct form of gene disruption operates when one or both breakpoints are located in heterochromatin. This "variegated position effect" (reviewed in Cattenach 1974, Spofford 1976) may operate over larger chromosomal segments, up to 50 polytene bands in Drosophila, but, in general, the loci effected are close to the breakpoint.

(iv) Meiotic Segregation

As is evident from the previous section, the meiotic behaviour of structural rearrangement is critical to their perceived role in chromosomal speciation. John (1981) defined four stages of meiosis that will determine the consequences of structural heterozygosity. These are:

(i) The pairing of homologous chromosome segments during zygotene which is dependant on the genetic content of the segments

(ii) crossing over between the paired segments during late pachytene

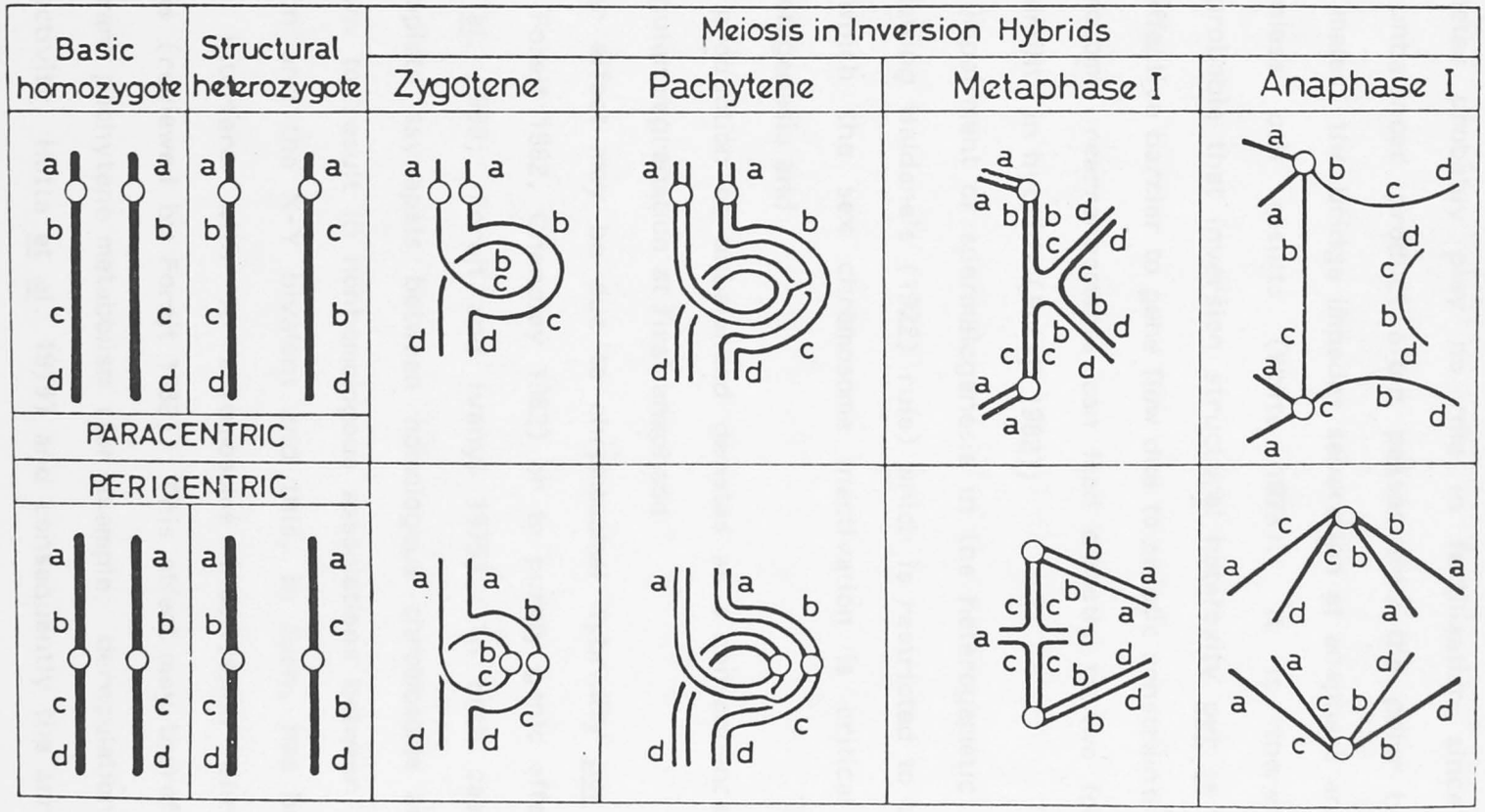
(iii) first metaphase orientation of the chromosomal associations determined by the chiasma formation in (ii) and

(iv) the segregation pattern of these chromosomes at first anaphase.

Together, these four stage will determine direct meiotic effects on fitness in the heterozygotes. A general discussion of the meiotic consequences of structural heterozygosity is given in Lewis and John (1963) and White (1973).

The degree of negative heterosis associated with both peri- and paracentric inversions is determined by the extent of homologous pairing and the distribution of chiasmata (White 1973). If reverse loops are formed at pachytene as a result of complete homologous pairing, a single chiasmata will lead to duplications and deficiencies in 50% of the gametes in the case of a pericentric inversion or a dicentric bridge and acentric fragment for a paracentric inversion (Fig. 1.1). There are however, several mitigating factors. In virtually all pericentric inversion heterozygotes studied to date, straight nonhomologous pairing occurs over the inverted region so that an inversion loop is not formed and recombination is suppressed (White 1973; John 1981; Coates and Shaw, in press). Similarly extensive nonhomologous pairing was observed in paracentric inversion heterozygotes within the Chironomidae (Martin 1967) although Nur (1968) reported a low frequency of inversion loops in

Figure 1.1. Meiotic segregation patterns in heterozygotes for paracentric (top) and pericentric (bottom) inversions, if synapsis is purely homologous, and recombination occurs within the inversion loop. Under these conditions, a dicentric bridge and an acentric fragment will be produced by a paracentric inversion heterozygote and a pericentric inversion heterozygote will produce 50% gametes with segmental duplications or deficiencies. Reprinted with permission from Lewis and John 1963.


Basic homozygote
Structural heterozygote
Meiosis in Inversion Hybrids
Zygotene
Pachytene
Metaphase I
Anaphase I
PARACENTRIC
PERICENTRIC

Camnula pellucida. Even when bridge and fragments are formed, the resultant gametes probably play no role in fertilisation since in females, the unbalanced product often passes into the polar body nuclei and in males, the bridge impedes separation at anaphase and a giant functionless cell results (White 1973). It is therefore considered improbable that inversion structural heterosity per se can constitute an effective barrier to gene flow due to meiotic constraints.

Interchromosomal rearrangements can lead directly to two forms of decreased fertility in hybrids (Forejt 1982);

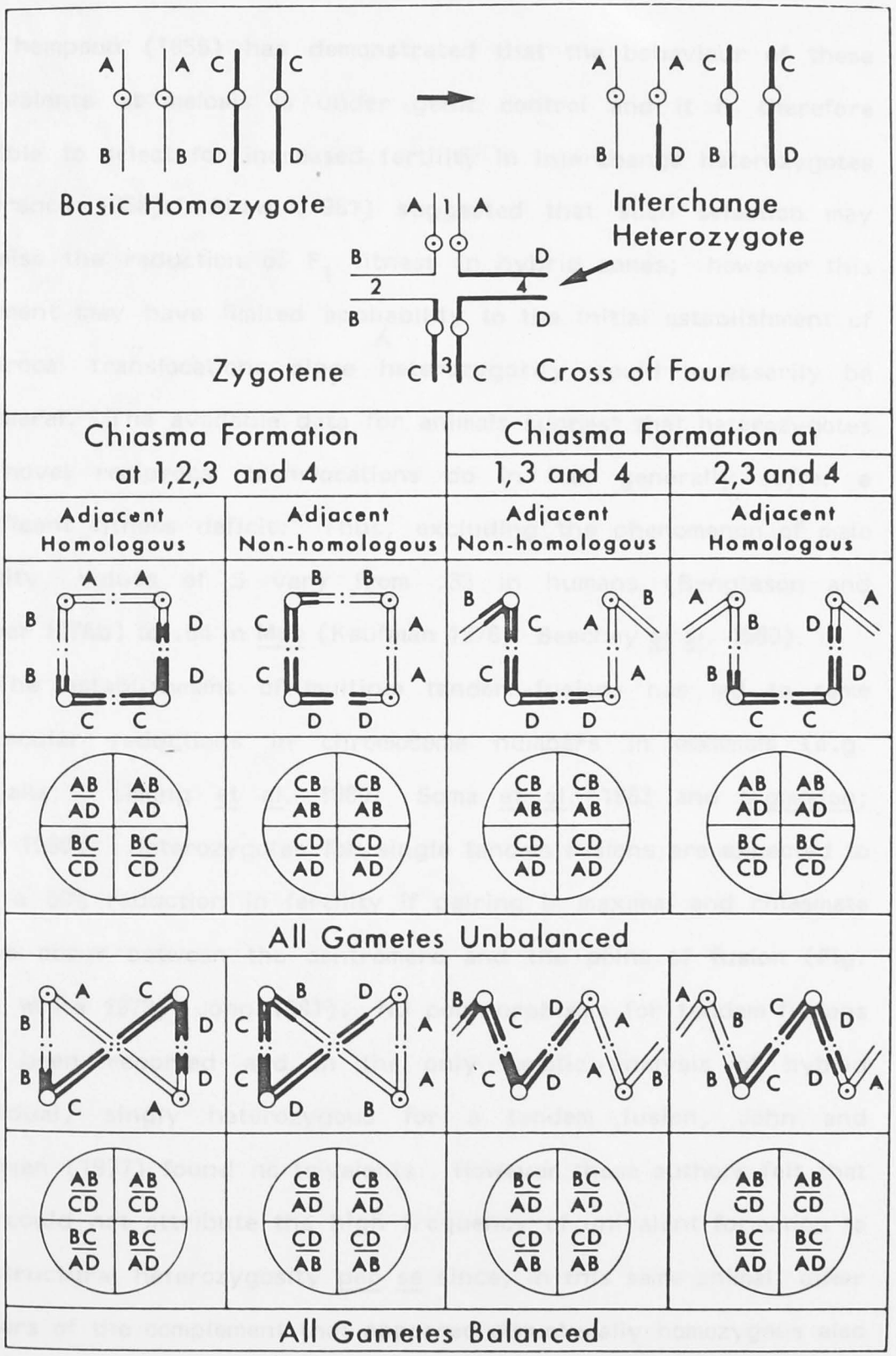
(1) the impairment of spermatogenesis in the heterogametic sex (following Haldane's (1922) rule) which is restricted to taxa in which the sex chromosome inactivation is critical in gametogenesis and

(2) the production of unbalanced gametes as a consequence of irregular segregation at first anaphase.

The former effect may be due to chromosomal hybridity per se (reviewed by Forejt 1982, Chandley 1982) or to purely genic effects (Chaganti et al. 1980; Forejt and Ivanyi 1975). In both cases, partial or complete asynapsis between homologous chromosome arms has been shown to result in nonhomologous associations between the unpaired region and the X-Y bivalent and this, in turn, has been suggested to interfere with X chromosome inactivation during spermatogenesis (reviewed by Forejt 1982). This effect may therefore result in aberrant pachytene metabolism (for example; deregulation of endonuclease activity, Hotta et al. 1979) and consequently the arrest of spermatogenesis at various stages (Chandley loc. cit., Forejt loc. cit.).

Of the various forms of balanced translocations, reciprocal translocations and tandem fusions appear to have the most serious meiotic consequences. In reciprocal translocation heterozygotes, the consequences are dependant on the number and position of chiasmata (Lewis and John 1963; Rickards 1983). For male sterile translocations (all X-autosome, and some purely autosomal translocations) the degree of spermatogenic arrest is positively correlated with the failure of chiasma formation in one or more arms of the quadrivalent (Fig. 1.2) (Chandley 1982; Forejt 1982). This effect, of course, does not operate in the female heterozygotes where fertility is determined by the segregation pattern. If chiasmata are formed between all four arms (Fig. 1.2) a ring orientation at first metaphase would inevitably result in all gametes being aneuploid, although in crosses between heterozygotes, this effect will be somewhat reduced at fertilisation by any complementation between unbalanced gametes (Stam 1979). If however the chiasmata are formed near the end of the arms then an alternate configuration leading to 100% disjunction may occur (Fig. 1.2 and White 1973). When only three chiasma are formed, a chain tetravalent results which may have either alternate (disjunctional) or adjacent (non-disjunctional) orientation (Fig. 1.2). In several plant and animal species permanent heterozygosity for reciprocal translocations has been established (reviewed by Grant 1981; John 1983) and in these cases alternate orientation at metaphase I predominates. Note that these cases generally involve metacentric chromosomes; reciprocal translocations involving telocentric or acrocentric chromosomes almost invariably lead to predominantly adjacent segregation (Lewis and John 1963).

Figure 1.2 The meiotic segregation patterns of quadrivalents formed in reciprical translocation heterozygotes. Only alternate segregation (bottom of figure) results in the production of balanced gametes. (Reprinted with permission from John 1976b).


Basic Homozygote
Interchange Heterozygote
Zygotene
Cross of Four
Chiasma Formation at 1,2,3 and 4
Chiasma Formation at
1, 3 and 4
2,3 and 4
Adjacent Homologous
Adjacent Non-homologous
Adjacent Non-homologous
Adjacent Homologous
All Gametes Unbalanced
All Gametes Balanced

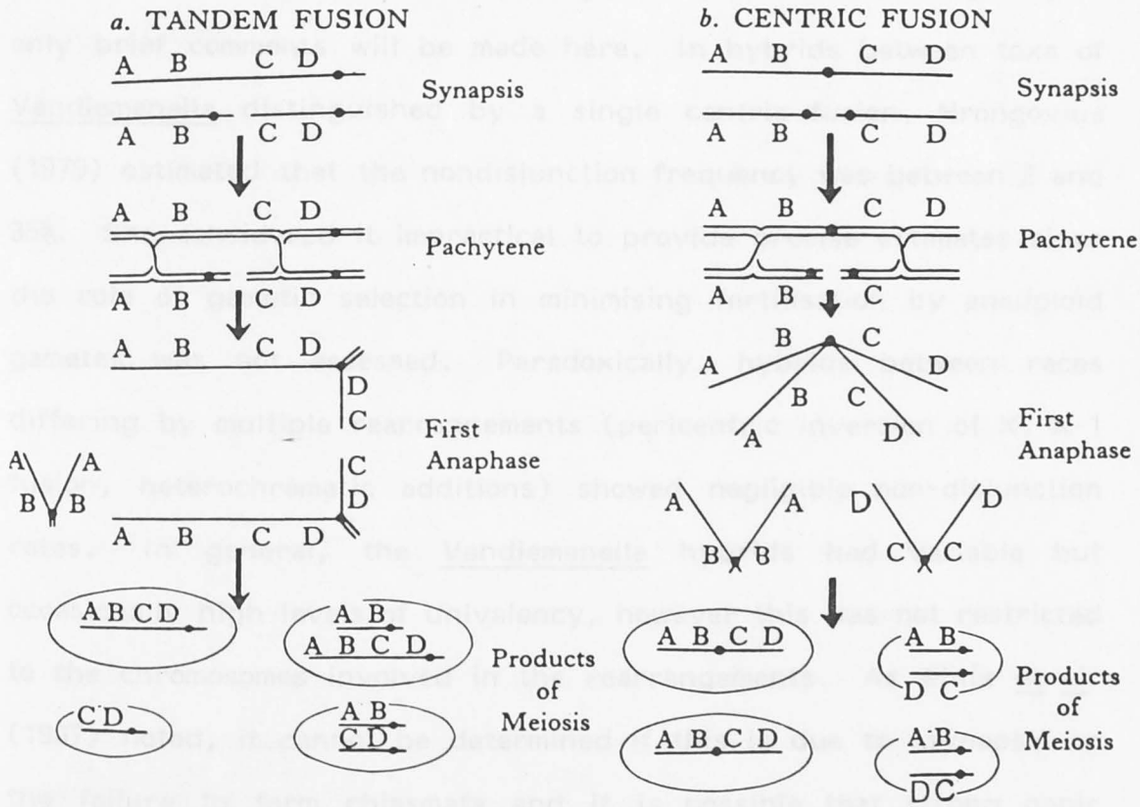
Thompson (1956) has demonstrated that the behaviour of these multivalents at meiosis is under genic control and it is therefore possible to select for increased fertility in interchange heterozygotes (Lawrence 1958). Shaw (1981) suggested that such selection may minimise the reduction of F_1 fitness in hybrid zones; however this argument may have limited appliability to the initial establishment of reciprocal translocations since heterozygosity would necessarily be ephemeral. The available data for animals suggest that heterozygotes for novel reciprocal translocations do in fact generally suffer a significant fitness deficit. Thus, excluding the phenomenon of male sterility, values of S vary from .33 in humans (Bengtsson and Bodmer 1976b) to .64 in Mus (Kaufman 1976; Beechey et al. 1980).

The establishment of multiple tandem fusions has led to some spectacular reductions in chromosome numbers in mammals (e.g. Muntjaks; Liming et al. 1980; Soma et al. 1983 and Sigmodon; Elder 1980). Heterozygotes for single tandem fusions are expected to have a 50% reduction in fertility if pairing is maximal and chiasmata always occur between the centromere and the point of fusion (Fig. 1.3a, White 1973; John 1981). No polymorphisms for tandem fusions have been reported and in the only meiotic analysis of hybrid individual, singly heterozygous for a tandem fusion, John and Weissman (1977) found no trivalents. However these authors felt that they could not attribute the high frequency of univalent formation to the structural heterozygosity per se since, in this same animal, other members of the complement that appeared structurally homozygous also displayed the same tendancy to form univalents.

Robertsonian translocations (= centric fusions or fissions) represent an extremely common form of polytypic variation in animals

and have been the subject of considerable controversy regarding their role as post mating barriers to gene flow (cf. White 1978a, John 1981, Shaw 1981; Patton and Sherwood 1983). In theory, a trivalent, formed by chiasmate associations between the fusion product and the ancestral chromosome arms may behave in one of two ways. Alternate orientation will lead to disjunction (fig. 1.3b) whereas if the centromeres of both the fusion product and one of the acrocentric chromosomes pass to the same pole (adjacent segregation), unbalanced gametes will result. To some degree, the frequency and position of chiasma will affect this process; proximal chiasmata are expected to increase the frequency of adjacent segregation (reviewed in Rickards, 1983). Since the meiotic behaviour of Robertsonian translocation heterozygotes is fundamental to the theories of stasipatry and chromosomal speciation, the available data will be examined in detail. However it is important to stress two points. First, when utilising data from interspecific hybrids it is difficult, though necessary to separate the fitness effects due to structural heterozygosity _per se_ and those attributable to differences in genetic background (John 1981; John _et al_. 1983; Shaw 1981; Patton and Sherwood 1983). Potent genic effects which reduce fertility in hybrids are widespread in plants (Grant 1981) and have been the subject of detailed study in _Drosophila_ (reviewed in Dobzhansky 1970) and _Mus_ (Forejt and Ivanyi 1975). Second, White (1982) has emphasised that it is not valid to evaluate the role of chromosomal speciation using examples that are clearly not representative of this process. Therefore, the taxa considered will be restricted to purported cases of chromosomal speciation.

Figure 1.3 (a-b) Meiotic segregation patterns in heterozygotes for a) tandem fusions and b) centric fusions assuming regular trivalent formation. In the former, 50% of gametes are expected to carry duplications or deficiencies. The centric fusion heterozygote (b) will produced balanced gametes if, as illustrated, segregation is disjunctional (see text). From White 1973.


a. TANDEM FUSION
b. CENTRIC FUSION
Synapsis
Pachytene
First Anaphase
Products of Meiosis
A B C D
C D

(i) Orthopterans. Grasshoppers of the Vandiemenella viatica group and stick insects belonging to Didymuria have frequently been cited as the best examples of stasipatric speciation (White 1973; 1978a, Bush 1975). In the former taxa meiotic analysis of F_1's was undertaken by White et al. (1967) and Mrongovius (1979) and Craddock (1974, 1975) reported similar studies for Didymuria. These data have been critically assessed by John (1981) and Shaw (1981) so only brief comments will be made here. In hybrids between taxa of Vandiemenella distinguished by a single centric fusion, Mrongovius (1979) estimated that the nondisjunction frequency was between 2 and 35%. She considered it impractical to provide precise estimates since the role of gametic selection in minimising fertilisation by aneuploid gametes was not assessed. Paradoxically, hybrids between races differing by multiple rearrangements (pericentric inversion of X, X-1 fusion, heterochromatic additions) showed negligible non-disjunction rates. In general, the Vandiemenella hybrids had variable but occasionally high levels of univalency, however this was not restricted to the chromosomes involved in the rearrangements. As White et al. (1967) noted, it cannot be determined if this is due to asynapsis or the failure to form chiasmata and it is possible that strong genic effects are operative (John 1981, 1983, and see Chaganti et al. 1980).

In Didymuria hybrids, Craddock (1975) found predominantly regular disjunction patterns where the races differed by two centric fusions but a sharp increase in univalency was observed for races distinguished by four such rearrangements. At the extreme (2n = 40 x 2n = 26, including tandem fusions, an X-autosome translocation and a neo-Y fusion), there were high levels of univalency and the formation of irregular higher multiples at first metaphase. A

perculiar feature of this analysis is that the observed frequency of univalents in natural hybrids (0-15%) was lower than for hybrids constructed in the laboratory (3.3-77.8%) (Craddock 1974, 1975) which suggests that selection to reduce non-disjunction is operating in the natural hybrid zones (see above). Once again, it appears probable that genic effects are acting to increase the infertility of these hybrids (White 1978a, Craddock 1974; John 1983).

(ii) Horses

The extensive chromosome variation observed between species and subspecies of the genus Equus has been interpreted as an example of stasipatric speciation (Bush 1981; Bush et al. 1977; White 1978a). These authors have repeatedly cited this group as an example of social structuring enforcing small deme formation in a highly vagile animal, yet Mayr (1982) and Charlesworth et al. (1981) emphasis the extent of gene flow caused by inter-herd movement of juveniles and young mares. Clearly, this matter requires further examination using ecological and biochemical techniques.

The karyotypic rearrangements separating the various Equus taxa are extremely complex and are not restricted to simple fusions (Ryder et al. 1978). In one case however, two forms are clearly separated by a single centric fusion. They are Przewalskis horse (2n=66) and the domestic horse (2n=64). In a hybrid between these forms, segregation of the fusion trivalent was normal and the F_1 male was therefore fully fertile (Short et al. 1974). In hybrids between the species which are separated by more complex multiple rearrangements, a meiotic block is apparent at pachytene of males which could be due to genic effects or structural heterozygosity (Chandley et al. 1975 and see above).

(iii) Rodents

Several species of rodents exhibit patterns of chromosome variation suggestive of chromosomal speciation (White 1978a). However sufficient data on meiosis in heterozygotes and F_1 fertility is available for only two of the genera - Rattus, and Mus.

In Rattus, hybrids between R. fuscipes (2n = 42) and R. lutreolus (2n = 38) appeared to be fully fertile (Fox and Murray 1979). On the other hand two geographical races of R. rattus which, as in the above case, differed by two independant fusions, (2n = 42 x 2n = 38) were semisterile. The reduced fitness of these F_1's is apparently attributable to genic effects (Baverstock et al. 1983a) since non-disjunction of the fusion trivalents was considered rare by Yosida (1980). A very different situation is apparent in hybrids between R. villosissimus (2n = 50) and R. colletti (2n = 42). A G-banding analysis showed that in addition to three independant fusions, the hybrids of this species display heterobrachial homology (Fig. 1.4) and therefore form a chain of five in addition to three trivalents at metaphase-I (Baverstock et al. 1983a). The F_1's between R. villosissimus and R. colletti exhibit a 70% reduction in fecundity which appears to be primarily attributable to the effects of structural heterozygosity since the genetic distance between them is negligible (Baverstock et al. loc. cit.).

The extensive analysis of the effects of heterozygosity for centric fusions in Mus musculus over the last 15 years has provided unparalleled information which has allowed the partitioning of the genic and cytogenetic components of negative heterosis. The major findings of this research are summarised in Table 1.2. The frequency of nondisjunction is invariably minimal in individuals singly

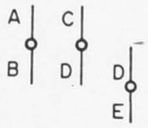
Figure 1.4 (A-C) Diagrammatic illustration of multiply heterozygous individuals with A) independant fusions; no arms shared, B) monobrachial homology; one arm shared and C) heterobrachial homology; two arms shared between fusions. The expected multiples for these classes of heterozygotes are shown on the right.

A. INDEPENDANT

IST MEIOTIC METAPHASE

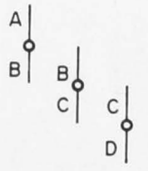
3 TRIVALENTS

B. MONOBRACHIAL HOMOLOGY



1 TRIVALENT, 1 QUADRIVALENT

C. HETEROBRACHIAL HOMOLOGY



1 PENTIVALENT

Table 1.2

A summary of the level of aneuploidy in the second meiotic metaphases in Mus that are heterozygous for centric fusions. With one exception, the "LAB" stocks are 2n = 40, all acrocentric as is "MUSCULUS". The LAB stock referred to in the last entry of the table has a single centric fusion that has monobrachial homology with POSCHIAVINUS. POSCHIAVINUS, CB and CD are the Italian multiple metacentric populations and are 2n = 28, 2n = 22 and 2n = 22 respectively. Note; (i) heterozygosity for single spontaneous centric fusions incurs negligible effects on fertility whereas if heterozygous metacentrics occur in a different genetic backround, the consequences are unpredictable. (ii) Mono- or heterobrachial homology in heterozygotes results in a severe disturbance of meiotic segregation.

TABLE 1.2

NUMBER AND ORIGIN OF HETEROZYGOUS METACENTRICS	% ANEUPLOIDY IN HETEROZYGOTES ♂	♀	REFERENCES
SPONTANEOUS MUTANTS IN LAB. STOCKS			
SINGLE	3-6		Gropp and Winking 1981
TRIPLE (INDEPENDANT)	21	26	White et al. 1978
FERAL METACENTRICS IN LAB. STOCK BACKROUND			
SINGLE	2-28	33-60	Gropp et al. 1982, Gropp and Winking 1981 Cattenach and Moseley 1973
DOUBLE (MONOBRACHIAL HOMOLOGY)*	31	57-74	Gropp in Capanna 1982, Gropp et al. 1972
INTERPOPULATION HYBRIDS			
POSCHIAVINUS x MUSCULUS (7 FUSIONS)	52	68	Capanna et al. 1976
CD x MUSCULUS (9 FUSIONS)	51	77	" " " "
CD x POSHIAVINUS } extensive mono-	+	92	" " " "
CD x CB } and heterobrachial	+		" " " "
CB x POSCHIAVINUS } homology	+		" " " "
POSCHIAVINUS x LAB (SINGLE MONOBRACHIAL FUSION)	58		Gropp et al. 1972

* Evans (1975) and Gropp and Winking (1981) found extensive male sterility in seven crosses of this type.

+ Male sterility.

heterozygous for spontaneous fusions that have arisen within laboratory stocks. However, the meiotic consequences become more severe as additional independant fusions are introduced. While White et al. (1978) did notice slight heterogeneity in nondisjunction frequency attributable to different strain backrounds, these data provide a relatively direct measure of the effects of structural heterozygosity per se.

Single fusions derived from feral population and introduced into a laboratory mouse background generally showed appreciably higher levels of nondisjunction but were extremely heterogeneous (Cattenach and Moseley 1973; Gropp and Winking 1981). It is now widely accepted that strong genic effects, presumably due to partial incompatibility between the genes in the feral metacentric and their homologues in the laboratory mouse contribute to the aneuploidy (Cattenach and Moseley loc. cit., Gropp and Winking loc. cit.). This is especially clear in comparisons of the observed aneuploidy with the frequency of post-implantation deaths. Baranov (1980) showed that the fertility of mice singly heterozygous for laboratory derived metacentrics was no less than that of homozygous controls despite having up to 10% trisomy amongst their embryos. In contrast, mice heterozygous for one feral metacentric show both slightly higher levels of nondisjunction and substantially elevated levels of post-implantation mortality. A particularly interesting feature of mice heterozygous for the feral metacentrics is that females almost invariably have higher non-disjunction rates that males (Gropp et al. 1982a).

Either heterobrachial or monobrachial homology dramatically increases the nondisjunction rate to the point where males are

generally sterile and the females are semisterile. Recent studies (Gropp et al. 1982a, Forejt 1982) have shown that male sterility occurs when multivalent chains are formed which have frequent non-homologous associations with the X-Y bivalent (see above). This association has not been observed in multiply heterozygous males which form multivalent rings (cf. chains) at first metaphase and accordingly, these do not show arrested spermatogenesis although they do suffer severely reduced fertility as a consequence of irregular segregation patterns (Gropp et al. 1982a). The significance of heterobrachial or monobrachial homology to the patterns of chromosomal speciation in Mus has recently been emphasised by Capanna (1982).

The interpopulation hybrids of Mus have yielded two results depending on the degree of hetero - or monobrachial homology. When M. poschiavinus (2n = 26) x laboratory Mus (2n = 40) or the "CD" M. musculus (2n = 22) x laboratory Mus hybrids were obtained, the males were semisterile and the females generally had higher levels of infertility (table 1.2). It is difficult to ascertain the genic component of the nondisjunction in these cases but it is notable that natural hybrid zones between all acrocentric and multiple metacentric populations are characterised by a high frequency of F_1 and backcross individuals (Capanna 1982; Spirito et al. 1980).

The laboratory produced hybrids between the various Robertsonian fusion populations were characterised by near-complete male and female sterility and in the few meioses obtained, ring multivalents were observed. Despite the frequent local sympatry between distinct multiple metacentric populations, natural hybrids have only rarely been detected between these forms and it is

therefore apparent that strong premating isolation is operative (Capanna 1982).

The available evidence in Mus does not suggest any gametic selection and the aneuploid gametes that do arise are therefore expected to effect embryonic survival. (Cattenach and Moseley 1973; Gropp and Winking 1981). In this respect mice contrast strongly with sheep in which aneuploid gametes are almost invariably eliminated before fertilisation (Bruere and Ellis 1979). One possible mitigating influence may be the iteroparous reproduction of Mus which has been suggested to reduce the consequences of aneuploidy by the rapid reproductive compensation for lost embryos (Bengtsson 1980; Lande 1979).

These meiotic data in Mus clearly demonstrate that in this species, heterozygosity per se for a single Robertsonian fusion has negligible effects on the fitness of hybrids. Gropp and Winking (1981) identified two forms of chromosomal sterility barriers that operate in Mus; (i) metacentrics introduced into different genetic backgrounds and (ii) multiple heterozygosity and especially where heterobrachial or monobrachial homology occurs.

When the data for all of these purported cases of chromosomal speciation are considered in conjunction, the inevitable conclusion is that animals heterozygous for a single Robertsonian fusion do not generally suffer a substantial impairment to fertility. Individual mosaics for novel mutants may represent an exception to this generalisation. In certain individuals of Valanga nigricornis (Teoh and Yong, 1983) and Atractomorpha similis (Peters 1982), some testicular follicles contained novel centric fusions and these follicles generated a high frequency of unbalanced gametes. However since

other follicles lacked the fusion and thus produced only balanced gametes it is not clear that these individuals would suffer from reduced fertility.

When at least one arm is shared between fusions in multiple heterozygotes the disruption of meiotic segregation is greatly enhanced. This observation has important implications for the stasipatric model of chromosomal speciation. As defined by White (1968, 1978a) single rearrangements were required to initiate the formation of a tension zone. While other types of rearrangements may satisfy the requirements for this process ($S > 0.3$; e.g. reciprocal translocations, tandem fusions), the fitness deficits characteristic of single Robertsonian fusion heterozygotes are far below that required. In fact, White himself recognised this fact when he proposed the chain process of stasipatry: "a single rearrangement does not entirely prevent introgressions of genes into the "fusion" population ... it may take as many as nine to reduce it to a negligible scale" (White 1978b, p.294).

1.4 Testing Models of Chromosomal Speciation

The mechanism(s) of speciation operating in any particular group of organisms must be inferred from the genetic and phenotypic properties of the extant taxa. It is therefore extremely difficult to determine the causative factor(s) in the speciation events since many of the observed changes may simply represent post-speciational divergence (White 1982).

In the preceeding discussion, three possible mechanisms of chromosomal speciation were evaluated. These are: (1) recombinational breakdown, (2) accumulation of structural changes

which individually have negligible effects on meiotic segregation in the heterozygous state, but which can act as effective barriers to gene flow if hybrids have hetero- or monobrachial homology and (3) single structural rearrangements resulting in a severe disturbance of meiotic segregation (chromosomal transilience, Templeton 1980b). To date, population genetic predictions have only been derived for the latter.

The small population sizes required for a chromosomal transilience are expected to lead to a variety of other genetic and phenotypic perturbations (see section 1.1). This has led several authors to claim that the establishment of chromosome changes are merely coincidental with the speciation process (Carson 1982, Wright 1982, Nei *et al*. 1983). or at best may make only a minor contribution to reproductive isolation (Templeton 1981). While these views may underestimate the role of chromosome changes in speciation (cf. White 1978a), they do emphasise that a correlation between chromosome change and speciation does not constitute proof that the rearrangements were the primary cause of the barrier to gene flow. The same argument, must of course apply to any other changes observed as a result of small population size.

Templeton (1980b) has derived qualitative predictions for the amount of structural gene divergence (genetic distance) that is expected to accumulate for different mechanisms of speciation. In principle, if the ancestral population structure can be inferred, then the most probable mechanisms of speciation can be identified from the level of genic differentiation between the incipient species. Under the most probable conditions for chromosomal transilience, Templeton (*loc. cit.*) predicted that the genetic distance between incipient species would be large. Small genetic distances would therefore

suggest that alternative mechanisms (including the other two categories of chromosomal speciation) were responsible for the speciation event. However it should be noted that the converse does not hold; larger genetic distances than those predicted may only reflect divergence subsequent to the speciation event.

In fact, the proposed examples of chromosomal speciation are almost invariably characterised by small genetic distances between ancestor-descendant chromosome races (Table 1.3). This observation does not, at first sight, appear compatible with the predictions for chromosomal transilience as defined by Templeton (1980b). However, if quantitative predictions are derived, then Templeton's predictions are seen to have little relevance to the taxa in question.

The theory reviewed in section 1.2 showed that the appropriate parameters for a chromosomal transilience are $S > 0.3$ and $N_e < 10$. Even if realistic levels of selection favouring the novel homozygote are allowed, the probability of fixation is still extremely low for populations with $Ne > 40$ (Hedrick 1981). The approximate estimates of the time to fixation within a deme, given in Table 1.4, were derived by substituting these values of S and N_e into Figure 2 of Lande (1979).

The number of generations for which N_e must remain low (Table 1.4) is small and while a strong reduction in heterozygosity may be expected (Chakraborty and Nei 1977), there would be insufficient time for significant genetic distance to accumulate as a result of mutation pressure. The expected genetic distances between the incipient species is therefore restricted by the initial heterozygosity of the ancestral population.

Table 1.3 Genetic distances (Nei's measure) within chromosome races and between ancestor-descendant pairs of chromosome races in species displaying extensive variation in chromosome number. Note that, although the genetic distances between races are low, they are not significantly lower than the maximum genetic distance predicted for a founder effect (see text).

TABLE 1.3

SPECIES	NO. OF LOCI	NO. OF PAIRWISE COMPARISONS BETWEEN RACES	AVERAGE GENETIC DISTANCE WITHIN RACES	AVERAGE GENETIC DISTANCE BETWEEN RACES (ancestor-descendant)	$\bar{H}$	MAXIMUM GENETIC DISTANCE ASSOCIATED WITH FOUNDER EVENT	REFERENCE
Sceloporus grammicus	19	3	.028	.030	max- .135 min- .039	.073 .020	Sites & Greenbaum 1983
Proechimys guairae	22	3		.030	.153 .060	.083 .031	Benado *et al.* 1979
Thomomys talpoides	31	3	.079	.036	.085 .008	.044 .004	Nevo *et al.* 1974
Spalax ehrenbergi	17	2		.030	.056 .018	.029 .009	Nevo & Shaw 1972
Rattus rattus	45	5	.053	.153	.06 .01	.031 .005	Baverstock *et al.* 1983b

Table 1.4 The approximate number of generations required for an underdominant rearrangement to reach fixation in demes of 10 and 50 individuals. Note that values of S below 0.1 will not, in theory, function as effective postmating barriers to gene flow (see text).

TABLE 1.4

		EFFECTIVE POPULATION SIZE		
		10	50	
	0.1	40	200	NO POST-MATING BARRIER
SELECTIVE DEFICIT OF HETEROZYGOTE RELATIVE TO HOMOZYGOTES (S)	0.3	26	130	POST MATING BARRIER
	0.5	20	100	POSSIBLE

Studies on the genetical structure of populations of rodents (Nevo et al. 1982c; Patton and Feder 1981; Patton and Sherwood 1983) and Sceloporus (Sites and Greenbaum 1983) suggest that a chromosomal transilience is only possible as a result of a founder event during colonisation or a severe reduction in population size. Chakraborty and Nei (1977) defined the maximum genetic distance between a large ancestral population and the founder population as $D_{max} = -[\log (1-H_o)]/2$ which is only obtained when the founder population has become completely homozygous. For the examples cited in Table 1.3, their small heterozygosities result in low D_{max} values and the observed distances either fall within the range of the predictions or exceed them. In general, the predictions given by Templeton (1980b) are not applicable to species with low heterozygosities (most invertebrates excluding Drosophila and nearly all vertebrates, see Nevo 1978) since "large" and "small" genetic distances cannot be effectively discriminated given the standard errors generally associated with this measure.

The absence of marked genic differentiation between the chromosomally distinct forms within these taxa has also been interpreted as evidence for gene flow between the races (Futuyma and Mayer 1980, Sites and Greenbaum 1983, Thaler et al. 1981). However, the extremely low genetic distances between R. villosissimus and R. colletti (Baverstock et al. 1983) indicate that reproductive isolation need not be reflected by structural gene divergence. It may be that the low genetic distances characteristic of the chromosomally distinct taxa are due to their relatively recent origin (White 1982).

It is evident that a broadly based approach must be adopted to make inferences on the mechanism of speciation from the patterns of variation amongst extant taxa (White 1978a). The minimal requirements include analysis of the ecology, habitat specificity, dispersal rates and genic, morphological and chromosome variation for the taxa in question. Complementary studies of natural hybrid zones and laboratory reared hybrids are also necessary but, unfortunately, in many organisms the latter approach is extremely difficult. For putative cases of chromosomal speciation, analysis of hybrid meiosis may allow definition of the effects of structural rearrangements on recombination and segregation.

1.5 The Organisms

Previous ecological and cytogenetic studies of Australian gekkonid lizards have suggested that they are suitable subjects for the study of chromosomal variation and its evolutionary consequences. The genera so far studied show extensive variation in chromosome number which indicates that Robertsonian fusions may be the predominant mechanism of chromosome change in the group (reviewed by King 1981). Two modes of chromosomal speciation have been suggested for these lizards; stasipatry in Diplodactylus vittatus (King 1977a) and primary chromosomal allopatry in Phyllodactylus marmoratus (King and Rofe 1976; King and King 1977) and Gehyra (King 1979; 1983, in press).

The principal aim of this thesis is to investigate the constraints upon chromosomal change and its role in speciation through a broadly based comparative study of two genera of Australian gekkonid lizards, Heteronotia and Gehyra. These organisms have several

characteristics which are ideally suited for such a study. Firstly, they have extremely widespread distributions patterns (Cogger, 1983) and their numerical abundance facilitates adequate sampling. Secondly, previous ecological studies by Bustard (1968a,b, 1969, 1970b) have demonstrated that their habitat requirements and home sites can be accurately defined. They are therefore especially suitable organisms for experimental studies of dispersal.

Unfortunately these geckos have long generation times and are therefore not amenable to experimental hybridisation during the time available for this study. Since sperm storage has been demonstrated in geckos (King 1977b); it is necessary to collect and isolate subadult females for the hybridisation studies. This did not allow sufficient time to produce mature F_1 hybrids for meiotic analysis. Consequently emphasis has been placed on detailed distributional studies complemented by ecological, electrophoretic, cytogenetic and morphological analysis.

On the basis of preliminary surveys of chromosome variation Heteronotia appeared to have a conserved chromosome number with minor variation in the number of chromosome arms. However, more extensive surveys revealed the existance of triploid parthenogenetic populations within this species. This discovery was then exploited in two ways. Firstly, this form of reproduction is intrinsically interesting and attention was directed towards determining the mode of origin and the extent of cytogenetic change occurring within the parthenogenetic lineages. Secondly, such systems represent a natural experiment in which the meiotic constraints on chromosome behaviour can be evaluated through extensive comparative studies of cytogenetic variation in the bisexual diploids and the effectively ameiotic

parthenogenetic triploids. The results of these studies, together with additional data from other parthenogenetic geckos and their bisexual relatives (Nactus, Lepidodactylus and Hemidactylus) are presented in chapter three.

The Gehyra variegata-punctata complex was selected for the study since it has been considered to represent a clear case of primary chromosomal allopatry (King 1979, 1981, in press). In chapter four, a detailed cytogenetic study employing chromosome banding techniques is presented. In addition to testing the suggested models of chromosome change derived from giemsa stained preparations (King 1979) attention was also directed towards evaluating the effect of habitat specificity on microgeographic differentiation.

In the course of these cytogenetic studies, a remarkable case of sex chromosome variation was discovered in Gehyra purpurascens. A detailed banding analysis of these variants is presented in chapter five. These data are related to current theories on the evolution of sex chromosomes and some consideration is also given to the possible reasons for the extreme variability of sex chromosomes relative to the autosomes of Gehyra.

In chapter six, the ecological studies of two chromosome races of Gehyra, which differ in their habitat specificity, are described. The analysis of dispersal patterns was facilitated by an independant series of replicated experimental removal studies of the same taxa. These ecological studies were complemented by analysis of the micro- and macrogeographic patterns of genic differentiation within and between the chromosome races of Gehyra.

Studies of speciation invariably concentrate on examples of incipient species or recently derived species. This has inevitably led to complications in demonstrating that the taxa studied are in fact valid biological species (see for example Key 1981, Futuyma and Mayer 1980). In chapter seven, the relationship between taxonomic and biological species is explored in Gehyra and a series of biological species is defined for the group. From this analysis, the cytogenetic electrophoretic and ecological data are applied in concert to evaluate the possible mechanism of speciation in Gehyra.

CHAPTER 2

TECHNIQUES

2.1 CYTOGENETICS

2.1.1 Chromosome preparations

Air-dried slides of mitotic metaphase and prometaphase cells were obtained from *in vitro* leucocyte cultures and *in vitro* bone marrow preparations. Of the two techniques, the former regularly yielded higher mitotic indices and generally provided mitotic metaphase spreads of superior quality. The *in vitro* bone marrow technique was therefore used only as a back-up for critical specimens, should the leucocyte cultures become contaminated or fail to show cell growth. This second problem was common in *Nactus*, and consequently the bone marrow technique was regularly employed for this species.

The culture media used in both techniques was modified from Thompson (1980) through the exclusion of antibiotics since these were found to significantly retard the rate of cell division. The ingredients of this medium are;

1. 80 ml Hams F10 medium
2. 20 ml Foetal Calf Serum - not inactivated
3. 2 ml Phytohaemaglutinin
4. 20 drops of Heparin from a 26g needle
5. L-Glutamine - final concentration of .6 mg/ml.
6. Concanavalin A - final concentration of 7 µg/ml.

After injecting the geckos intracranially with Nembutal, sterile blood samples were collected from the chest cavity of each individual.

From one to ten drops of whole blood were added to 5 ml of the culture medium and these tubes were then maintained at 35°C for 70 to 80 hours. During this time, the cells were resuspended by inversion, at least twice a day.

These cultures were harvested by the addition of 0.1 ml of .001% colchicine to each culture for one hour at 35°C. After centrifugation at 1400 rpm for five minutes, the supernatant was discarded and the cell pellet resuspended in .075 M KCl for 20 to 30 minutes. The cells were then spun down and resuspended in four changes of 3:1 methanol/acetic acid fixative; the first of which was of at least a 20 minute duration. Slides were prepared by first dropping cell suspensions from a pasteur pipette onto slides covered with a film of distilled water, and then air drying the slides. It was found that cell suspensions in fixative could be stored at -4°C for up to a year and still provide suitable material for the chromosome banding analyses.

The in vitro bone marrow preparations were obtained by a technique similar to that described by Christidis (1983). Even in juvenile geckos, sufficient bone marrow could be obtained by dissecting out the spinal column, placing it in a shallow bath of culture medium (see above) and gently tapping it with a brass rod until a cell suspension was formed. After removing the remaining bone, muscle and nervous tissue, the cell suspension was immediately treated with colchicine (1 drop of a .001% solution) for 40 minutes. Longer periods of exposure to colchicine were found to reduce the quality of the metaphase spreads. The hypotonic and fixation treatments were the same as those previously described.

Meiotic preparations were obtained without colchicine treatment. Testes were teased apart with fine forceps in .075 M KCl, and then vigorously aspirated in a pasteur pipette to produce a homogeneous cell suspension. The subsequent hypotonic and fixation treatments were identical to those used for mitotic preparations. Although this procedure yielded suitable diakinetic and metaphase cells, anaphase was never observed. Presumably, the method of homogenising the tissue, together with the procedure for making air-dried slides, disrupts the anaphase spindle.

2.1.2 Staining and banding procedures

Giemsa stained karyotypes. Air-dried slides were left at room temperature for at least one hour and then stained with 10% Giemsa in a pH 6.4 phosphate buffer for four minutes. The slides were then rinsed in the phosphate buffer and two changes of distilled water and drained. The karyotypes produced by this method are variously described as gross, standard or giemsa stained karyotypes in this thesis.

C-banding. A hot $Ba(OH)_2$ method similar to that described by King (1980) was employed. Slides were first aged by overnight incubation at 40-50°C and then pretreated in 0.2N HCl for 20 minutes and rinsed thoroughly. They were then treated with a saturated (5%) $Ba(OH)_2$ solution at 45°C for 25 to 40 seconds, thoroughly rinsed, and placed in 2 x SSC at 65°C for one hour, rinsed again and stained with 10% Giemsa for 15 to 20 minutes. To obtain good results it was necessary to remove $BaCO_3$ from the $Ba(OH)_2$ solution by allowing it to stand for at least two hours before use. When chromosome

preparations appeared darkly stained after C-banding, the procedure was repeated with the exclusion of the HCl pretreatment.

N-banding. The N-banding method was modified from Gerlach (1977). Air dried slides were placed on a hot plate (approx. 50°C) for one hour and then immersed in a pH 4.2 1M NaH_2PO_4 solution at 95°C for 5 minutes. The slides were then removed, rinsed thoroughly and stained with 10% Giemsa for 10 minutes. This procedure was used most extensively for G. purpurascens (chapter 5). In this species, a large interstitial N band was present on the Z chromosome under the experimental conditions described above. However, when the hot NaH_2PO_4 treatment was extended to 7 minutes or more, the only area that still stained with Giemsa was the NOR on chromosome 10.

Silver staining. Olert's (1979) modification of the silver staining technique was used. Three drops of a solution, made by adding 1 drop of 0.2% formic acid (adjusted to pH 4.2 with sodium formate) to 7 drops of 50% silver nitrate, were placed on one day old slides. A coverglass was then added and the slide incubated for 10 minutes in a moist chamber held at 50°C. The slides were then carefully and thoroughly rinsed with distilled water and drained. The best results were obtained with a 12 to 18 hour old 50% silver nitrate solution.

G-banding. G-banding was obtained through the modified trypsin-versene technique described by Leversha et al. (1980). Air dried slides were aged by either incubating them at 50°C overnight, or placing them in a 20% by volume solution of hydrogen peroxide for

20 minutes and then rinsing in distilled water. These slides were then exposed to a 1% Trypsin (Difco) solution in Hanks basal salt (without Ca^{2+} or Mg^{2+}), supplemented with 1% by volume 10X Versene (see Leversha et al. 1980 for Versene ingredients). The required treatment time was found to vary between 5 and 30 seconds. Slides were then rinsed in Hanks basal salt, 70% ethanol and 90% ethanol, air dried and stained with a 5% Giemsa solution for 5 minutes. It was frequently necessry to retreat slides one or more times before satisfactory G-banding was obtained. For each of the banding methods described above and G-banding in particular, the most critical requirement was to have a large number of metaphase spreads with elongate chromosomes and close chromatid apposition.

In situ hybridisation. The insitu hybridisation of an 18 + 28S ribosomal DNA probe, derived from Drosophila, onto gekkonid metaphase cells was conducted by Dr. R. Honeycutt and Ms. N. Conteras. The technique employed is described by White et. al. (1982).

2.2 ELECTROPHORESIS

Electrophoresis was performed on liver and muscle extracts prepared from tissues that had been stored at -170°C for up to three years. With few exceptions, every specimen analysed by electrophoresis had also been karyotyped.

The tissue extracts were obtained by grinding either muscle or liver in an equal volume of a pH 7.0 solution consisting of .1M Tris, .001M EDTA, .005 M NADP with 5 µl of ß mercaptoethanol per 10 ml of solution (Selander et al. 1971). The homogenates were spun in an Eppendorf centrifuge for five minutes and the supernatant (excluding

fat) was collected for electrophoresis. The supernatants were stored at -170°C for the duration of the electrophoretic analyses.

Electrophoresis was performed using cellulose acetate gels (Cellogel, Chemitron Italy) as the support medium. Before loading the individual samples onto the gels with a drafting pen, the gels were removed from the 30% methanol storage solution, floated on the surface of the soaking buffer for 10 minutes and then fully immersed in this buffer for at least 20 minutes.

The running and staining conditions appropriate to each protein system analysed in Gehyra and Nactus are described in Table 2.1. These conditions were selected for their ability to separate electromorphs, and in some cases, the resolution of each band was reduced to achieve this end. At the beginning of each study, all protein systems were run on each of the commonly employed buffers (TM, TEM, TEB, TC - see Table 2.1) to determine the optimum conditions.

Some examples of gels from the studies of Gehyra and Nactus are shown in Figure 2.1. For protein systems with more than one band of activity, the most anodal band was designated as the first locus. A genetic basis for the variation in the electrophoretic phenotypes of a given locus was assumed but could not be demonstrated since, (i) the sample sizes were too small to allow for a test of fit between observed genotypic proportions and those expected under Hardy-Wienberg equilibria and (ii) it was not possible to conduct controlled inheritance studies. Several protein systems were excluded from the analyses since the observed patterns of variation could not be sensibly interpreted on the basis of the known phenotypes for monomeric or multimeric enzymes (described in Harris and Hopkinson,

TABLE 2.1

Running and staining conditions for protein systems assayed by electrophoresis in Gehyra and Nactus. Tissue abbreviation: L = liver, M = muscle. Numeric codes for buffers;

1. TEM, 50mM TRIS, 5mM EDTA, 1mM $MgCl_2$, pH 7.8 with MALEIC acid
2. TEM, 15mM TRIS, 5mM EDTA, 1mM $MgCl_2$, pH 7.8 with MALEIC acid
3. TEB, 15mM TRIS, 5mM EDTA, 1mM $MgCl_2$, pH 8.2 with BORIC acid
4. TC, 100mM TRIS, pH 8.2 with CITRIC acid.
5. PHOS, 20mM PHOSPHATE, pH 7.0.

Cofactors (NAD^+ or $NADP^+$) - 10 mg added to soaking and running buffers.

* all gels run at 10-12 V/cm at 4°C.

** assayed for Gehyra only.

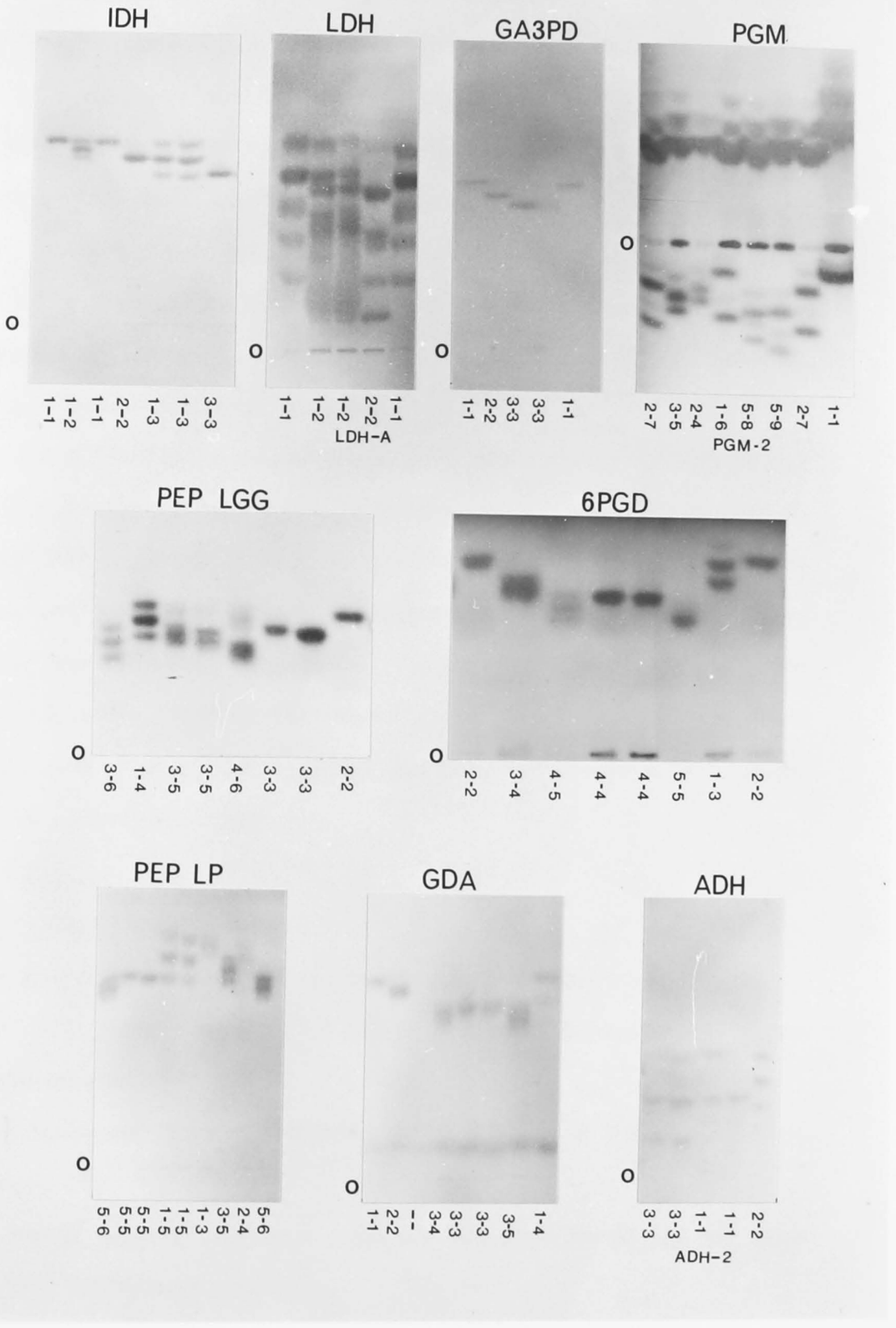
*** assayed for Nactus only.

TABLE 2.1

PROTEIN SYSTEM, abbreviation used in text and EC number	TISSUE	Number of loci	RUNNING BUFFER & CONDITIONS*	STAIN
Aconitase (ACON) E.C. 4.2.3.1	L	2	1, 1½ hrs.	Richardson, 1983
Adenylate Kinase (AK) E.C. 2.7.4.3.	M	1	1, 2 hrs	Richardson et al. 1980
Alanine amino transferase (GPT) E.C. 2.6.1.2	L	1	4, 1 hr.	Modified from Harris & Hopkinson 1976
Alcohol dehydrogenase (ADH) E.C. 1.1.1.1	L	1-2	Gehyra: 1 with NAD^+, 3 hrs Nactus: 3 with NAD^+, 3 hrs	Richardson, 1983
Aspartate amino transferase (GOT) E.C. 2.6.1.1	L	2	4, 2 hrs	Richardson et al. 1980
Fumarase (FUM) E.C. 4.2.1.2	L	1	4 with NAD^+, 3 hrs.	Richardson et al. 1980
Fructose-biphosphate aldolase (ALD) E.C. 4.1.2.13	M	1	1 with NAD^+, 3hrs	Richardson et al. 1980
General protein (GP)	M	2-5	1, 3 hrs	Gartside 1972
Glucose phosphate isomerase (GPI) E.C. 5.3.1.9.	M	1	Gehyra: 1, 3 hrs Nactus: 3, 2½ hrs	Richardson et al. 1980
Glyceraldehyde-phosphate dehydrogenase (α GPD) E.C. 1.2.1.12	L	1	Gehyra: 1 +NAD^+, 3 hrs Nactus: 3+ NAD^+, 2 hrs	Richardson et al. 1980
Glycerol-3-phosphate dehydrogenase (GA3PD) E.C. 1.1.1.8	L	1	Gehyra: 1+ NAD^+, 3 hrs. Nactus: 3+ NAD^+, 2hrs	Richardson et al. 1980
Guanine deaminase** (GDA) E.C. 3.5.4.3	L	1	Gehyra: 1, 1 hr	Richardson 1983
Hexokinase*** (HK) E.C. 2.7.1.1	M	1	1 with $NADP^+$, 2 hrs	Richardson 1983

Isocitrate dehydrogenase (IDH) E.C. 1.1.1.42	L	1-2	4, 2 hrs	Richardson et al. 1980
Lactate dehydrogenase (LDH) E.C. 1.1.1.27	L	2	Gehyra: 1, 3 hrs Nactus: 3, 3 hrs	Richardson et al. 1980
Malate dehydrogenase** - NAD^+ (MDH) E.C. 1.1.1.40	L	2	1 with NAD^+, 3 hrs	Richardson et al. 1980
"Nothing" dehydrogenase - $NADP^+$ (NDH)	L	1-2	1 with $NADP^+$, 2 hrs	1.0 ml pH 8.2 Tris HCl, 5 mg $NADP^+$, 0.1 ml 5M $MgCl_2$, .1 ml PMS (2 mg/ml), .1 ml MTT (4 mg/ml)
Mannose-phosphate isomerase (MPI) E.C. 5.3.1.8	L	1	Gehyra: 2, 1 hr Nactus: 1 with $NADP^+$, 30 min	Richardson 1983
Peptidase D, substrate - L-leucyl proline (PEP LP) E.C. 3.4.13.9	L	1	Gehyra 1, 1½ hrs Nactus 3, 1½ hrs	Richardson 1983
Peptidase - substrate = L leucylglycylglycine (PEPLGG) E.C. 3.4.11	L	1	1, 1½ hrs	Richardson 1983
Phosphogluconate dehydrogenase (decarboxylating) (6PGD) E.C. 1.1.1.44	L	1	1 or 3 with $NADP^+$ 1½ hrs	Richardson et al. 1980
Phosphoglucomutase (PGM) E.C. 2.7.5.1	L	2	2, 2 hrs	Richardson et al. 1980
Phosphoglycerokinase*** (PGK) E.C. 2.7.2.3	M	1	4, 1½ hrs	Richardson 1983
Phosphopyruvate hydratase (ENO) E.C. 4.2.1.11	M	1-2	5, 3 hrs	Richardson 1983
Pyruvate kinase (PK) E.C. 2.7.1.40	M	1	1, 2 hrs.	Richardson et al. 1980
Superoxide dismutase (SOD) E.C. 1.15.1.1	L	1	ANY	Richardson et al. 1980
Triose-phosphate isomerase (TPI) E.C. 5.3.1.1	M	1	1 with NAD^+, 3hrs	Modified from Harris & Hopkinson 1976.

Figure 2.1 Examples of electrophoretic phenotypes and their respective interpretations. In some cases electromorphs exhibited very slight mobility differences but were conservatively scored as representing the same 'allele'. For example, the '3' alleles in the two '3-5' heterozygotes shown on the Pep LGG gel were scored as identical since it would not be possible to unambigously differentiate the alternative homozygotes from heterozygotes should there be any variation in activity. The ADH loci presented complex phenotypes in the case of Nactus. The formation of heterodimers between loci was observed in all samples from some populations but not was observed other populations. In the gel shown, heterodimer formation is evident in the first, second and fifth samples. The fourth sample has no activity at the ADH-1 locus.


IDH
LDH
GA3PD
PGM
O
1-1 1-2 1-1 2-2 1-3 1-3 3-3
1-1 1-2 1-2 2-2 1-1
LDH-A
1-1 2-2 3-3 3-3 1-1
2-7 3-5 2-4 1-6 5-8 5-9 2-7 1-1
PGM-2
PEP LGG
6PGD
3-6 1-4 3-5 3-5 4-6 3-3 3-3 2-2
2-2 3-4 4-5 4-4 4-4 5-5 1-3 2-2
PEP LP
GDA
ADH
5-6 5-6 5-5 5-5 1-5 1-5 1-3 3-5 2-4 5-6
1-1 2-2 -- 3-4 3-3 3-3 3-5 1-4
3-3 3-3 1-1 1-1 2-2
ADH-2

1976). These included; Esterases, Peptidase (substrate = L-leucyl alanine) and Purine-nucleoside phosphorylase. In these cases, the number of bands was not related to the age of the samples and it is most probable that the observed patterns of variation reflected overlapping loci that varied independantly. An $NADP^+$ dependant nothing - dehyrogenase (NDH) was detected on several gels for which the $NADP^+$ cofactor was added to the soaking and running buffers. When all other substrates were removed from the staining solution, one or two bands of activity appeared.

The data from these experiments were analysed using the BIOSIS computer package for electrophoretic data (D.L. Swofford and R.K. Selander, University of Illinois at Urbana, U.S.A., 1981). Nearly all statistics and electrophoretic phylogenies presented were obtained by analysing the observed genotypic arrays with this program. The standard error estimates for Nei's (1978) genetic distance were calculated with a program supplied by Dr. J. Daly from the CSIRO Division of Entomology, Canberra.

2.3 MORPHOMETRICS

The method of taking measurements of head dimensions in Gehyra (chapter 7) followed King (1984). The definitions of these measurements are;

1. Snout-vent length: distance from the tip of the rostral scale to the vent.

2. Snout length: distance from the anterior margin of the orbit to the tip of the rostral scale.

3. Head length: distance between the anterior margin of the ear orifice and the tip of the rostral scale.

4. Head width: distance between the lateral surfaces of the head measured at the angle of the jaw.

5. Head depth: distance between the dorsal and ventral surfaces of the head measured at the angle of the jaw. The dorsal colour and pattern were subjectively assessed from specimens that had been stored at -20°C. No individuals that had been preserved in alcohol or formalin were used since this procedure is known to cause a rapid loss of colour and, at the extreme, may obsure even marked back patterns (King 1984).

The data were analysed in the simplest manner consistent with the objectives (see chapter 7). The regression coefficients between the head dimension ratios and their denominator, head length, were calculated using the SPSS statistical package. The same program was employed for the approximate t-tests and calculations of means and standard errors.

CHAPTER 3

PARTHENOGENESIS IN THE GEKKONIDAE

3.1 The theory

The evolution and maintenance of sexual reproduction presents a formidable problem for evolutionary biologists. The widely accepted "cost of sex" argument proposes that sub- or asexual forms will maintain up to a two-fold selective advantage over their sexual competitors since they are not required to produce males (review by Maynard Smith 1978; Lloyd 1980). Further, sexual populations are expected to suffer from recombinational load, i.e. the disruption of adaptive gene combinations by recombination, and may be disadvantaged by the requirement to find a mate (reviewed by Williams 1975; Maynard Smith loc. cit., Bell 1982). This latter effect may be particularly important in low density populations of sexual organisms (Gerritson 1980). In the face of these assumed constraints on sexuality, why is it that only about one in a 1000 animal taxa (White 1978a) have alternative mechanisms of reproduction?

Before reviewing the theoretical advantages of sexual reproduction and the predictions that emerge from the hypothesis that have been proposed to account for the prevalence of sexual reproduction, it is necessary to clarify some of the terminology that will be used in this discussion (Table 3.1). Sexual reproduction is any process that combines together normal meiosis and fertilisation. Asexual reproduction refers to the substitution of mitotic divisions for meiosis in the absence of fertilisation. Subsexual organisms have a modified meiosis of some sort, may or may not require fertilisation, and reproduce by a number of distinct processes. These may be

Table 3.1

Classification of the various reproductive mechanisms that are discussed in the text. In the top section, emphasis has been placed on the mechanism of oogenesis or proliferation. The predicted effects on heterozygosity, in the absence of selection, are shown on the right. The automictic mechanisms may lead to immediate homozygosity, fixed heterozygosity and intermediate conditions depending on the restitution mechanism employed. In the lower part of the table, subsexual reproductive modes are categorised by their requirement for male gametes and the ultimate fate of sperm.

TABLE 3.1

Modes of Reproduction	Details of Reproductive Process				Effect on Heterozygosity
		Meiotic			
	Mitotic	Normal	Modified	Fertilisation	
1. Asexual	+	-	-	-	Increase with mutation
2. Sexual	-	+	-	+	-
3. Subsexual					
a. Apomictic	-	-	+ Replaced by mitotic type mechanism	± May be hybridogenetic, gynogenetic or parthenogenetic	Increase with muation
b. Automictic	-	-	+ Restitution by intra- or post-meiotic fusion or DNA replication prior to diplotene	± as for apomictic subsexuality	May increase or decrease depending on restitution mechanism employed - see text.

FATE OF MALE GAMETES

Subsexual Mechanism	
1. Parthenogenetic	Sperm not required
2. Gynogenetic	Sperm required for initiation of development but do not contribute to the zygote
3. Hybridogenetic	Paternal genome expressed in progeny but is excluded from germ line prior to oogenesis.

defined according to the role of male gametes. The term parthenogenesis literally means virgin reproduction and in this discussion will be restricted to situations in which the production of an embryo from a female gamete in totally independant from the participation of male gametes. In contrast, both gynogenesis and hybridogenesis are sperm dependant. In the former, sperm penetration of the oocyte is required to initiate development but the sperm subsequently degenerates or is extruded and so makes no genetic contribution. Alternatively, hybridogenesis refers to the process where haploid eggs are fertilised by the sperm and although the male genome is expressed in the progeny, it is eliminated from the germ line prior to oogenesis and only the maternal genome enters meiosis.

When considering the relative merits of sexual and subsexual reproduction (asexuality is unknown in vertebrates) the important distinction concerns the precise meiotic mechanism involved since these vary in their potential for recombination and thus their genetic consequences (Table 3.1). Apomictic reproduction has a mitotic like phase and is therefore expected to conserve the genotype with the exception of alterations due to mutation (White 1970, 1978a). The extent and consequences of recombination in modes of subsexual reproduction that maintain some form of meiosis (automictic) are dependant on the timing of restitution.

In some cases the somatic ploidy level is known to be maintaned by an additional chromosome replication (Bell 1982). The precise time at which this "meiotic reduplication" occurs is rarely known with certainty. It is frequently claimed that an additional DNA replication occurs in the last premeiotic mitosis (e.g. White et al. 1963; Cuellar

1971) but this suggestion is only based on the number of bivalents observed at later stages of meiosis. However recent studies of the meiosis of parthenogenetic stick insects (Koch et al. 1972; Pijnacker and Ferwerda 1978, 1982) and gynogenetic Ambystoma (Sessions 1982) have provided evidence for an extra round of DNA replication during pachytene. This is turn requires a desynapsis of paired homologues and a resynapsis involving the sister-products of the extra round of replication. Under these conditions, synapsis and recombination are assumed to be restricted to the identical molecular copies which leads to total genomic conservation in the absence of mutation (White 1970, 1978a). However, rare recombination events between non-identical homologues could result in a gradual approach to homozygosity at a rate depending on the frequency of such recombination, the subsequent orientation of multivalents at first metaphase and the magnitude of selection against homozygotes (Asher and Nace 1971).

In general, these heterozygosity-maintaining mechanisms are the only ones compatible with odd numbered ploidy levels such as triploidy (White 1970, 1978a), and in fact the vast majority of apomicts and automicts with meiotic reduplication are polyploid (Bell 1982). An immediate advantage of polyploidy is that it allows a rapid increase in functional heterozygosity due to both the higher frequency and tolerance of mutations relative to that in comparable diploids (Lokki 1976a,b). An alternative mode of maintaining the somatic ploidy level in automicts is intrameiotic restitution which can occur by several means. The rate of loss of genetic variation will vary according to the precise mechanisms involved and also depends on the frequency of recombination between the locus and the centromere (details in Asher 1970). In this respect, it is important

to note that it is possible to maintain heterozygosity at overdominant loci closely linked to the centromere (Asher and Nace 1971). By contrast, post-meiotic restitution, which is achieved by an extra chromosome replication in the first zygotic cleavage division, is expected to immediately enforce total homozygosity (Asher 1970, White 1970, 1978a).

The extraordinary diversity of models that have been proposed to account for the predominance of sexual reproduction have been reviewed and refined by Williams (1975), Maynard Smith (1978) and Bell (1982). It is beyond the scope of this discussion to provide more than a cursory examination of this voluminous theory. In broad terms, the rarity of subsexuality has been attributed to evolutionary restrictions on its occurrence (historical arguments) or to its failure to become established in competition with pre-existing sexual species (advantages of sex hypotheses). In this context it should be noted that there are really two questions to be considered; (1) how did sex first evolve and (2) how is it retained (White 1978a). Only the second question is pertinent to this study since the empirical evidence to be presented concerns examples of secondarily evolved subsexuality.

(i) The Historical Hypothesis

The historical argument has been advanced primarily by Williams (1975) who suggested four barriers that must be simultaneously overcome for stabilised subsexual reproduction to occur. Together, these may be considered as a potential cost of parthenogenesis which will, to some extent, balance the cost of sex (see above). These are (1) the required alterations to the meiotic process, (2) the avoidance

of fertilisation by sympatric bisexual individuals, (3) the initiation of regular development and (4) the avoidance of homozygosity and inbreeding depression.

White (1978a) suggested that the transition from sexual reproduction to parthenogenesis required a macromutation of some sort involving simultaneous changes at a number of loci and emphasised that strong selection would act against intermediate meiotic phenotypes. In view of the proposition that multiple loci are involved in the required meiotic alterations (White 1978; Marshall and Brown 1981; Templeton 1982b) it is a disappointing and restrictive feature of all of the models discussed below that they only consider the evolution of subsexuality from a sexual population in terms of allelic changes at single locus.

As Bell (1982) and White (1978a) stress, there is an urgent requirement for the genetic mechanisms involved in the origin of parthenogenesis to be identified. The available data indicate that subsexual reproduction may originate as a consequence of hybridisation or spontaneously (tycho- parthenogenesis) although the relative contribution of these two mechanisms remains a matter of debate (Bell 1982). It is generally accepted that automictic mechanisms with intra- or post-meiotic restitution are achieved via tychoparthenogenesis (White 1978a; Bell 1982; Templeton 1982b) and it has also been proposed that apomixis is then secondarily derived from such automictic states (Suomalainen et al. 1976; Templeton 1982b, but see White 1973 for a contrary view). However hybridisation between genetically distinct taxa may be important in the origin of automictic organisms with meiotic reduplication (White 1970, 1978a; Uzzell 1970) although some authors (Cuellar 1974, 1978; Maynard Smith 1978) do not find the evidence compelling.

The insemination of an unreduced egg produced by a parthenogenetic organism will result in a triploid which is expected to be infertile unless it maintains meiotic reduplication or is apomictic (White 1978a). The requirement that organisms with a newly acquired parthenogenetic capacity avoid insemination may consequently impose ecological and possibly behavioural constraints on the evolution of subsexuality (reviewed by Cuellar 1977). With regard to the third restriction proposed by Williams (1975), there is virtually no information on the molecular requirements for the initiation of regular development in the absence of sperm.

The avoidance of homozygosity and subsequent inbreeding depression is critical to the establishment of both parthenogens with post-meiotic restitution and automictic parthenogenetic mechanisms that enforce homozygosity (Williams 1975; Maynard Smith 1978). This effect is evidenced by the poor viability of tychoparthenogenetic birds (Markert 1982) and orthopherans (Bell 1982) which are characterised by post-meiotic restitution. Further, Nur (1971) reported a correlation between the establishment of homozygosity enforcing automictic reproduction and the occurrence of haploid males (arrhenotoky) in the related species of coccids. This suggests that this form of parthenogenesis is most readily evolved from a population in which recessive deleterious mutations are already exposed to selection. Paradoxically, attempts to establish stable parthenogenetic lines with post-meiotic restitution in Drosophila are less likely to succeed with inbred lines. This has led to the suggestion that the initial intense selection for viability is principally due to the requirement for coadaptation rather than the elimination of recessive lethals (Templeton 1982b).

Whatever the cause, it is clear that the viability of progeny produced by parthenogenesis, and by tychoparthenegenesis in particular, is frequently lower than for related bisexual organisms (Lamb and Willey 1979; Lloyd 1980; Bell 1982; Templeton 1982b but see Schall 1978). Since the equivalance of fecundity in competing sexual and subsexual females is a critical assumption of the cost of sex argument (Maynard Snith 1978) it follows that the postulated two fold selective advantage of subsexual reproduction may not be realised in some taxa.

Despite his acceptance of a historical argument to account for the precise mode of subsexuality adopted, Bell (1982) discounts this restriction as a general explanation for the rarity of subsexual reproduction on the philosophical grounds that, given its ubiquity, sex must be, in some way, adaptive. However, he does propose two predictions from the historical hypothesis. Firstly, on the assumption that subsexuality is not readily evolved, this mode of reproduction should be taxonomically concentrated rather than conforming to particular ecological conditions. Secondly, that obligate subsexual lineages should have the some meiotic mechanisms as their facultative or tychoparthenogenetic progenitors. The latter prediction allows no role for a hybrid origin which Bell (loc. cit.) considers to be of little significance to the evolution of subsexual reproduction.

(ii) The postulated advantages of sex

The search for an explanation for the maintenance of sex based on either long term group selection or short term individual selection has produced a series of hypotheses for which the essential features and emergent predictions are summarised in Table 3.2. Most of these

Table 3.2

A synopsis of the theories that have been proposed to account for the maintenance of sexual reproduction (see text). This summary excludes the historical hypothesis which is presented in the text. Note that many of the models differ principally in emphasis (cf. Hitch Hiker and Fisher-Muller; Tangled bank and Red Queen). The predictions have been primarily derived from Bell (1982).

TABLE 3.2

Model	Basis and environmental context	Predictions
1. Mullers Ratchet	Long term accumulation of deleterious mutations in subsexual organisms - group selection	1. Higher frequency of deleterious mutants in old subsexual lineages. 2. Low frequency of deleterious mutants in automicts with intrameiotic or post meiotic restitution.
2. Hitch-Hiker	Reduced efficiency in response to selection in subsexual organisms due to total linkage. Extinction of clones in fluctuating environements.	1. Subsexual populations should have poor response to selection and should be rare in fluctuating environments.
3. Fisher-Muller	Absence or recombination retards rate of evolution by preventing rapid association between new mutations - long term group selection relying on extinction of clones in fluctuating environemnts	1. Subsexual populations should have high extinction rates in fluctuating environments. 2. Subsexual populations should be depauperate in genetic variation
4. Best Man	Extreme trunctation selection such that only rare recombinant genotypes reproduce - short term individual selection. Operates only in capricious environments.	1. As for Model 3 2. Subsexual populations should have low clonal diversity.
5. Differential-Extinction	Subsexual populations are overspecialised to narrow niche. Destruction of niche causes extinction. Operates in temporally fluctuating and spatially heterogeneous environments.	1. As for Model 3 2. Subsexual populations should have restricted ecological flexibility relative to bisexual populations.
6. Tangled Bank	Narrow ecological amplitude of subsexual clones prevents utilisation of multiple niche environments. Reduced genetic correlation in sexual forms decreases inter-sib competition. Clones become extinct through sampling error or niche destruction.	1. Subsexual organisms should predominate in novel or disturbed habitats where competition is minimised. 2. In spatially heterogeneous environments, total density will increase with clonal diversity. 3. In sympatry, sexual and subsexual forms occupy distinct niches.
7. Red Queen	Subsexual populations cannot co-evolve with competitors, prey, predators, pathogens or parasites. Frequency dependant selection with time lag creates capricious environment and and causes clonal extinction. Emphasis on biotic interactions.	1. Subsexual organisms predominant in novel or disturbed habitats. 2. Rare in regions of strong biotic stress including complex species rich communities.

emphasise the purportedly narrow genetic and ecological flexibility of parthenogenetic populations and predict that subsexual clones will become extinct as a result of spatial and/or temporal environmental heterogeneity.

One model that does not invoke environmental changes as a causative factor is Mullers ratchet. This suggests that, in the long term, a given subsexual clone will accumulate deleterious mutations and thus an enhanced mutational load since these mutations cannot be lost through selection against the homozygotes produced by recombination (Muller 1964). This basic model has been elaborated by Manning (1983) and Kondrashov (1982) who suggest that the group selection advantage for sexuality is maximal for a large number of mutations which individually are only slightly deleterious (see also Maynard Smith 1978 who proposes that this will only operate in small populations). Factors that may reduce the effect of Mullers ratchet include polyploidy, which may provide a buffering effect for the accumulation of mutations (Lokki 1976b) and epistatic fitness interactions, which may increase the efficiency of selection against deleterious mutations in subsexual populations (Bell 1982). Further, Lynch and Gabriel (1983) have argued that most deleterious mutations will be masked in the case of polygenic characters.

The hitch-hiker model (reviewed by Maynard Smith 1978) emphasises the effect of linkage in reducing the response to directional selection at a single locus. Thus, in the absence of recombination, selection between clones for an advantageous allele is restricted by any selective forces acting on other loci within the genome. Thompson (1976) suggests that this effect will only be important in small populations with few loci and strong linkage

disequilibrium. A general prediction of the model is that parthenogenetic populations will fail to respond to temporal fluctuations in the environment and will therefore become extinct under these conditions (Table 3.2).

The concept of the reduced rate of evolution in the absence of recombination has been attributed to Fisher and Muller. However, as Bell (1982) notes, this concept in fact predates Mendelian genetics. The Fisher-Muller model (renamed the "Vicar of Bray" by Bell) suggests that recombination allows rare advantageous mutants to be combined in a single individual at a greater rate than is possible under the conditions of subsexual reproduction. This hypothesis complements Mullers ratchet, is similar to the hitch-hiker model (see above) and shares with these, the requirement for group selection. However as Maynard Smith (1971) demonstrated, sexuality will only be advantageous for this reason in populations larger than 10^6 since in smaller populations, the preceeding mutation will have become fixed by selection and the advantage of recombination is lost. Further, the models require multiplicative fitness and any positive epistasis will remove the advantage of sex. Templeton (1982b) has emphasised the effect of the change in the unit of selection which accompanies the transition from sexual reproduction to subsexuality. He argues that, under the latter condition, selection will operate on non-additive and non-multiplicative fitness interactions between loci which in effect may allow a very rapid response to selection. Finally, Lynch and Gabriel (1983) have modeled the mutation-selection balance for a polygenic system and found that subsexual populations could potentially evolve at a sufficient rate to account for observed dynamics of phenotypic evolution. These recent theoretical studies appear to undermine the

previously widely accepted notion that subsexual lineages are incapable of adaptive change and thus represent evolutionary dead ends.

The remaining models represent attempts by Williams (1975), and Bell (1982) in particular, to produce viable individual selection theories for the maintenance of sexual reproduction. Williams (1975) and Bell (1982) propose that severe truncation selection will result in only rare recombinants of high fitness surviving to reproduce in a given generation (the best-man hypothesis, Table 3.2). An alternative but related suggestion is that, in fluctuating environments, the least fit genotype may fail to reproduce in one generation and if this occurs in a subsexual lineage, that genotype cannot be replaced by recombination. (Williams 1975). Both of these mechanisms can only operate effectively in highly fecund organisms subject to intense selection pressures which led Williams (1975) to despair of ever finding an adaptive interpretation of sex in low fecundity organisms. Maynard Smith (1971) demonstrated that environmental fluctuation is not a sufficient condition to maintain sex under this model; instead, sex will only be favoured by selection when the sign of correlation between significant environmental variables changes between generations. This form of temporal environmental heterogeneity, called "capricious" (Bell 1982), was considered unrealistic by Maynard Smith (1971). However when the effects of biotic interactions are considered, it becomes more plausible (see the Red Queen hypothesis below).

The hitch-hiker, Fisher-Muller and best man models discussed above share the assumption that subsexual populations are incapable of a rapid response to selection in temporally heterogeneous environments and would consequently have a high rate of extinction.

Williams (1975) and Thompson (1976) have achieved a conceptual volte-face in proposing a differential extinction hypothesis (Table 3.2) that predicts the extinction of subsexual clones in a spatially heterogeneous environment due to their overspecialisation to a narrow and temporally unstable niche. They argue that sexual populations evolve slower than their parthenogenetic competitors due to the continuing perturbation of the genome by recombination. Note that this effect is precisely the same as recombinational load which is also regarded as a cost of sex (see above). In a spatially heterogeneous habitat, the sexual population will consequently have broader ecological amplitude and the sporadic elimination of any one niche would not be detrimental to the whole population whereas a highly specialised subsexual clone would be eliminated.

A closely related hypothesis attributable to Bell (1982), considers the consequences of one or a few ecologically restricted clones competing with a broad-niched sexual population in a complex environment. Bell (loc. cit.) envisages an equilibrium situation under these conditions, with the balance between subsexuality and sexuality determined by the ecological flexibility of the combined clones in any one habitat. Further, he suggests that the consequences of intersib competition would be decreased for sexual populations due to their lower genetic correlation (see also Lloyd 1980). In this case sexuality will be favoured if clonal diversity is reduced by sampling effects (in small populations) or if temporal variation causes extinction of overspecialised clones as suggested by Williams (1975) and Thompson (1976). Bell (1982) felt that this hypothesis could not account for the maintenance of sexual populations in competition with subsexual forms with moderate diversity (or single clones with wide ecological

amplitude) and reasonable reproductive efficiency. The same restrictions would apply to the similar differential-extinction hypothesis (Table 3.2) and the former requirement may be violated by Templeton's (1982b) suggestion that the interactive nature of fitness effects of subsexual clones would lead to broad ecological tolerances.

A final class of models, which consider the effects of biotic interactions beyond intraspecific competition, have been collectively termed the Red Queen hypothesis by Bell (1982) and may provide the capricious environmental changes envisaged in the best man model. This hypothesis considers the effects of frequency dependant and time lagged selection pressures characteristic of coevolution in predator-prey interactions and host-pathogen relationships (Jaenike 1978; Glesener and Tilman 1978; Lloyd 1980; Bell 1982). The essence of these models is that a subsexual population lacks the genotypic and/or ecological flexibility to respond to opposing selection pressures between generations. This seems an intuitively powerful argument when the rapid adaptation of parasites and pathogens with short generation times relative to their host is considered. It receives empirical support from the observation of increased susceptibility to pathogens in genetically homogeneous plant cultivars (Marshall 1977). As a variant on this theme, Tooby (1982) suggested that if pathogens are genotype specific, the greater genotypic diversity of sexual populations alone would lead to the maintenance of sexual reproduction. From these arguments, the emergent prediction (Table 3.2) is that parthenogenesis should be viable in habitats with a reduced potential for pathogens such as temporally unstable habitats or isolated island populations (Jaenike 1978). However, this prediction should be applied with caution as the pathogen load on

subsexual versus sexual populations is a function of both the species diversity of pathogens and their relative virulence. More generally, parthenogenesis should predominate in areas of low biotic (as opposed to physical) stress (Glesener and Tilman 1978; Bell 1982), such as areas of predictable physical adversity (Greenslade, 1982).

This brief review of the various hypotheses for the maintenance of sex and their emergent predictions (summarised in Table 3.2) suggest four complementary areas of research that are urgently required and empirically feasible. These are:-

(1) genetic analysis of the mode of origin of parthenogenesis from previously sexual taxa, which should ideally incorporate analyses of naturally occurring parthenogenesis, coupled with laboratory studies on the experimental induction of subsexuality in normally sexual forms;

(2) documenting the genetic diversity within parthenogenetic forms and apportioning this variation between the component present at the origin of subsexuality and that which has arisen by subsequent mutation. Note that in the case of a hybrid origin, this approach requires a comparison of the variation present within the putative parental taxa to that found in the derived parthenogen. The implicit assumption in a study of this sort is that, given adequate sample sizes, all variants present in the parental taxa at the time of origin of the parthenogen can still be detected in extant bisexual populations;

(3) determining the environmental correlates of parthenogenesis in taxa where direct comparison with the ecological distribution of the progenitor sexual relatives is possible and;

(4) relating these broad patterns to comparative studies of the niche breadth and reproductive capacity of these subsexual and sexual populations with emphasis on sympatric populations.

In this chapter, a series of cytogenetic studies on parthenogenetic gekkonid lizards will be presented which aim to present direct information on some of these areas of research. In accordance with the principles outlined above, the general aim of these studies was to ascertain the mode of origin of parthenogenesis, the cytogenetic diversity within the parthenogenetic lineages and the broad environmental correlates of parthenogenesis in these organisms. The proposed laboratory studies of the genetic mechanisms of origin of parthenogenesis and the desirable, but time consuming, comparative ecological and reproductive studies could not be achieved within the time constraints of the study.

Of the taxa to be considered, the most substantial data base concerns Heteronotia binoei. By comparison the information available for Nactus arnouxii, Lepidodactylus lugubris and Hemidactylus is much more restricted. These data will be considered in two contexts. First, with regard to the maintenance of sexuality in vertebrates and second, in relation to the constraints on the establishment of chromosome change (see section 1.3). In the virtual absence of recombination, apomictic and certain classes of automictic parthenogens should be released from the meiotic constraints operating on the establishment of chromosome rearrangements, which in turn, leads to the prediction of increased karyotypic diversity in such subsexual lineages (White 1975).

3.2 Case studies

3.2.1 Heteronotia

The genus Heteronotia is endemic to Australia and consists of two currently recognised species; H. spelea which is restricted to north-western and possibly central Australia (Pilbara, Great Sandy desert, Tanami desert) and the more widely distributed H. binoei (Kluge 1963; Cogger 1983). The latter species occurs throughout continental Australia with the exception of the extreme south-eastern and south-western regions (Cogger loc. cit., Fig. 3.22). Zoogeographic analysis suggests that this genus is a relatively recent element of the Australian herpetofauna and entered the continent from Torres Strait (Cogger and Heatwole 1981).

The ecology of H. binoei in the western deserts has been studied by Pianka and Pianka (1976) who emphasised the broad habitat tolerances and dietary requirements of this terrestrial species. Opportunistic prey selection was also reported for H. binoei under relatively mesic conditions in northern New South Wales (Bustard 1968b).

(i) Diploid Heteronotia (2n = 2x = 42) - Standard karyotypes.

The results of an extensive analysis of the geographic pattern of variation in the standard karyotypes (N = 266 individuals) and C-banding patterns (N = 138 individuals) of diploid bisexual H. binoei are summarised in Table 3.3. The strategy employed was to subdivide the widespread distribution of H. binoei into a series of geographically defined cytotypes on the basis of their standard karyotypic variants and then to further resolve each cytotype into a series of karyomorphs distinguished by their C-banding patterns.

Table 3.3

Localities from which diploid Heteronotia were analysed cytogenetically. Sample sizes are shown for both standard karyotypes and C-banding. The numbering of the localities (1-57) refers to points on the distribution maps -figs. 3.2 and 3.4. It was possible to identify a series of geographically defined groups of populations based on their standard karyotypes (CYTOTYPES) which could be further resolved into a series of KARYOMORPHS on the basis of their C-banding patterns. On the right hand side, the presence (+) or absence (-) of a distal secondary constriction on chromosome 6 is noted.

TABLE 3.3

LOCALITY	STANDARD CYTOTYPE (N)	C. BAND KARYOMORPH	(N) ♂	(N) ♀	SECONDARY CONSTRICTION
A. H. binoei					
1. Atherton QL.	A6 (1)	CYA6	1		+
2. Mapoon mission QL.	A6 (3)	CYA6	1	1	+
3. 80 km W Barry Caves N.T.	A6 (1)	EA6	1		+
4. 100 km E Mt. Isa QL.	A6 (1)	EA6		1	+
5. Wyandra QL.	A6 (2)	EA6	1	1	+
6. 11 km N Baradine N.S.W.	A6 (3)	EA6	1	1	+
7. Macquarie Marshes N.S.W.	A6 (1)				+
8. 40 km SW Maree S.A.	A6 (4)	EA6	2		+
9. Coonatie W/H. S.A.	A6 (1)			1	+
10. Moolawatana Stn. S.A.	A6 (1)			1	+
11. Rawlinna Stn. W.A.	A6 (4)	EA6	2	2	+
12. Mungara Stn. W.A.	A6 (2)	EA6	1	1	+
13. MacDonnell Ranges* N.T.	A6 (66)	CA6	20	18	-
14. Kulgera* N.T.	A6 (9)	CA6		2	-
15. Native Gap N.T.	A6 (2)	CA6	1	1	-
16. Cordillo Downs Stn. S.A.	A6 (4)	CA6	1	1	-
17. Thundelarra Stn.* W.A.	A6 (1)	CA6**		1	-
18. Tallering Stn.* W.A.	A6 (1)	CA6**		1	-
19. Woomarel Stn. W.A.	A6 (6)	CA6**	1	4	-
20. Hamelin Stn. W.A.	A6 (2)	CA6**	1	1	-
21. Yoothapinna Stn. W.A.	A6 (1)	CA6**		1	-
22. Billabong R/H W.A.	A6 (3)	CA6**		1	-
23. Carbla Stn. W.A.	A6 (4)	CA6	1	2	-
24. Kalbarri W.A.	A6 (1)				-
25. Jabiluka N.T.	A6-2 (5)	NA6	2		+
26. Baroalba N.T.	A6-2 (4)		1	2	+
27. Bing Bong Stn. N.T.	A6-2 (3)	NA6	1	2	+
28. Berrimah Darwin N.T.	A6-2 (3)	NA6	1	1	+
29. Hayes Ck. N.T.	A6-2 (4)	NA6		1	+
30. Mataranka N.T.	A6-2 (4)	NA6	2	2	+
31. Wyndham W.A.	A6-2 (4)	WYA6	2		+
32. Doongan Stn. W.A.	A6-2 (2)	KA6		2	+
33. Drysdale River Stn. W.A.	A6-2 (1)	KA6		1	+
34. Mitchell Plateau W.A.	A6-2 (3)				+
35. Nita Downs Stn. W.A.	SM6-1 (8)	SM6-1a	2	3	+
36. Bamboo Ck. W.A.	SM6-1 (6)	SM6-1a	1	1	+
37. Sandfire Flats W.A.	SM6-1 (2)				+
38. Carrarang Stn. W.A.	SM6-1 (6)	SM6-1b	2	2	+
39. Endearby Isl.* W.A.	SM6-2 (2)	SM6-2b		1	+
40. Glenayle Stn.* W.A.	SM6-2 (6)	SM6-2a	3		+
41. Turkey Ck. W.A.	SM6-2 (5)	SM6-2b	1	2	+
42. Halls Ck. W.A.	SM6-2 (4)	SM6-2b		1	+
43. Notabilis Hill W.A.	SM6-1 (1)				+
44. Mt. Beadell W.A.	SM6-1 (1)				+
45. Ayers Rock/Mt. Olga* N.T.	SM6-1 (3)	SM6-2a	1	1	+
46. The Granites, N.T.*	SM6-1 (1)	SM6-2b	1		+
47. Tea Tree* N.T.	SM6-2 (7)	SM6-2b	1		+
48. Wauchope N.T.	SM6-2 (4)	SM6-2a	2	1	+
49. Barrow Ck. N.T.	SM6-2 (3)	SM6-2b	1		+

TABLE 3.3 (cont.)

LOCALITY	STANDARD CYTOTYPE	C. BAND KARYOMORPH	(N) ♂	(N) ♀	SECONDARY CONSTRICTION
50. Frewena R/H N.T.	SM6-2 (3)	SM6-2b	1		+
51. Renner Springs N.T.	SM6-2 (1)	SM6-2b		1	+
52. Soudan Stn. N.T.	SM6-2 (2)	SM6-2b	1		+
53. Camooweal QL.	SM6-2 (5)	SM6-2b	1	1	+
54. Daly Waters N.T.	SM6-3 (2)	SM6-3a	1	1	+
55. Willeroo N.T.	SM6-3 (3)	SM6-3a	2	1	+
56. Karratha W.A.	SM6-3(2)	SM6-3b	1	1	+
57. Narwtarra* W.A.	SM6-3(3)	SM6-3b	1	1	+
B. H. spelea					
36. Bamboo Ck. W.A.	A6 (1)	SPELEA		1	+
C. H. sp. nov.					
13. MacDonnell Ranges					
Ross River Stn.	A6-3 (8)	SP. NOV.	1	1	-
Undoolya Stn. 1.5 km E	SM6-4 (16)	SP. NOV.	4	5	-
Alice Springs	SM6-4 (1)	SP. NOV.			-
Bond Spring Stn.	SM6-4 (3)	SP. NOV.	1		-

* Sympatric with triploid parthenogens

** polymorphic or fixed for procentric band on pair 4.

The diploid Heteronotia karyotype invariably consists of 42 chromosomes however the number of chromosome arms shows geographic variation, the most significant of which concerns chromosome pair 6 (Fig. 3.1). Within H. binoei, four polytypic or polymorphic variants of this chromosome were identified from standard karyotypes (Fig. 3.1H) and these form the basis for the primary subdivision of the diploids into a series of cytotypes.

The widely distributed A6 cytotype has no short arms on chromosome 6 (Figs. 3.1B, D) and may be further subdivided into two geographic components distinguished by the presence or absence of a distal secondary constriction on chromosome 6. (Figs. 3.1Hd and 3.1He). Populations from eastern and southern Australia expressed this secondary contriction (e.g. Fig. 3.1B) whereas the A6 populations from central and western Australia didn't (Figs. 3.1D, 3.2). A silver staining analysis of individuals from these latter populations revealed that, despite the absence of a secondary constriction, an active nucleolar organising region (NOR) was maintained in the terminal region of chromosome 6 (Fig. 3.3).

Populations of H. binoei from north-central and north-western Australia had a morph of chromosome 6 with minute short arms and a distal secondary constriction (Fig. 3.1A, C, Hg). This morph was polymorphic with the A6 variant (Fig. 3.1Hd, 3.1A) in the north-central populations but was fixed in samples from north-western Australia (see Fig. 3.2). All populations in which this form of chromosome 6 was observed have been designated as the A6-2 cytotype (Table 3.3).

This A6-2 morph of chromosome 6 is quite distinct from the submetacentric chromosome 6 that defines the SM6 group of cytotypes

Figure 3.1 (A-H) Polymorphic and polytypic variants observed amongst standard karyotypes of bisexual H. binoei. In all cases the arrow defines the location of the distal secondary constriction on chromosome 6.

(A) an A6-2 individual from Baroalba (N.T.) heterozygous for short arms on chromosome 6.

(B) the A6 cytotype typical of eastern, southern and some northern populations.

(C) an A6-2 individual from Bing Bong (N.T.) heterozygous for short arms on chromosome 1.

(D) the A6 cytotype representative of central and western populations. Note the absence of a secondary constriction on chromosome 6. This individual from Cordillo Downs (S.A.) was also heterozygous for short arms on chromosome 4.

(E) a SM6-2 individual from Barrow Ck (N.T.) heterozygous for short arms on chromosome 4.

(F) the SM6-2 cytotype found in north-central Australia. Note the short arms on chromosome 4.

(G) the SM6-3 cytotype found in areas of northern and western Australia. Note that the short arms of chromosome 4 are smaller than in the SM6-2 cytotype (cf. G and E and F).

(H) Comparison of the morphology of the variants of chromosomes 4 and 6 respectively. a) the telocentric morph of chromosome 4. b) The acrocentric morph that defines the SM6-2 cytotype (see E,F). c) The acrocentric morph that defines the SM6-3 cytotype (see G). d) The telocentric morph of chromosome 6 with a secondary constriction (see B). e) the telocentric morph without a secondary constriction that is characteristic of central and most western A6 populations (see D). f) The morph of chromosome 6 that defines the SM6 group (see E,F,G). g) The variant that is characteristic of the A6-2 cytotype (see A,C).

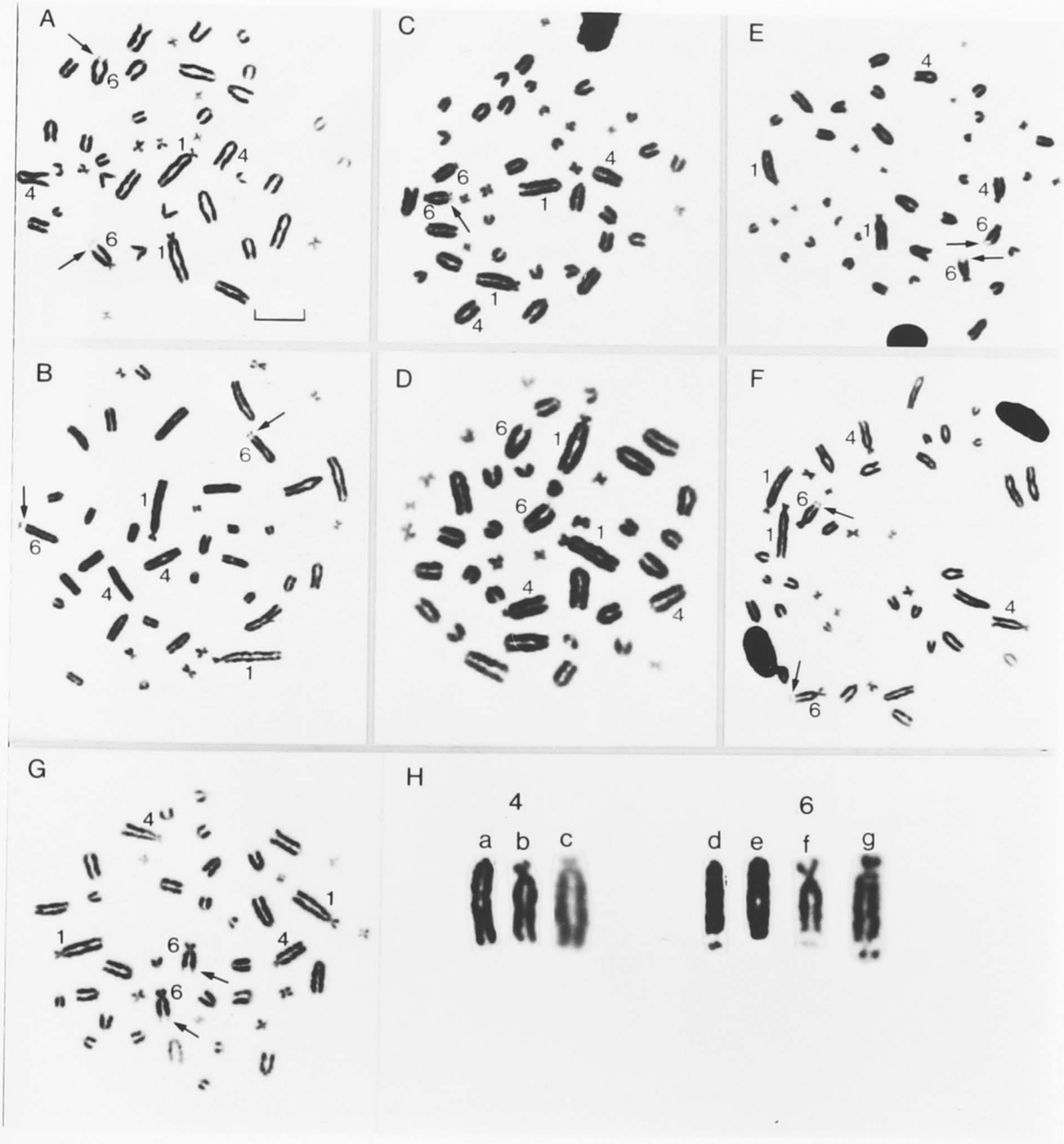

A
B
C
D
E
F
G
H
4
6
a b c
d e f g

Figure 3.2 The geographic distribution of the various karyomorphs of the A6 and A6-2 cytotypes. State borders and the tropic of Capricorn are shown. The numbers refer to table 3.2. The dotted line encloses the known distribution of the SM6 cytotypes (see Fig. 3.4). Note that there are three areas of overlap between the A6 and SM6 groups; the central Northern Territory (3), the Shark Bay region on the Western Australian coast (17-24) and Bamboo Ck (36). The only case of sympatry was at Bamboo Ck where H. spelea (A6) and H. binoei (SM6-1a) occurred together.

Symbols :

- □ CA6
- ■ CYA6
- ○ EA6
- ● SPELEA

A6 CYTOTYPE

- ▽ NA6
- ▲ KA6
- △ WYA6

A6-2 CYTOTYPE

- \+ A6 with secondary contriction no C-banding.

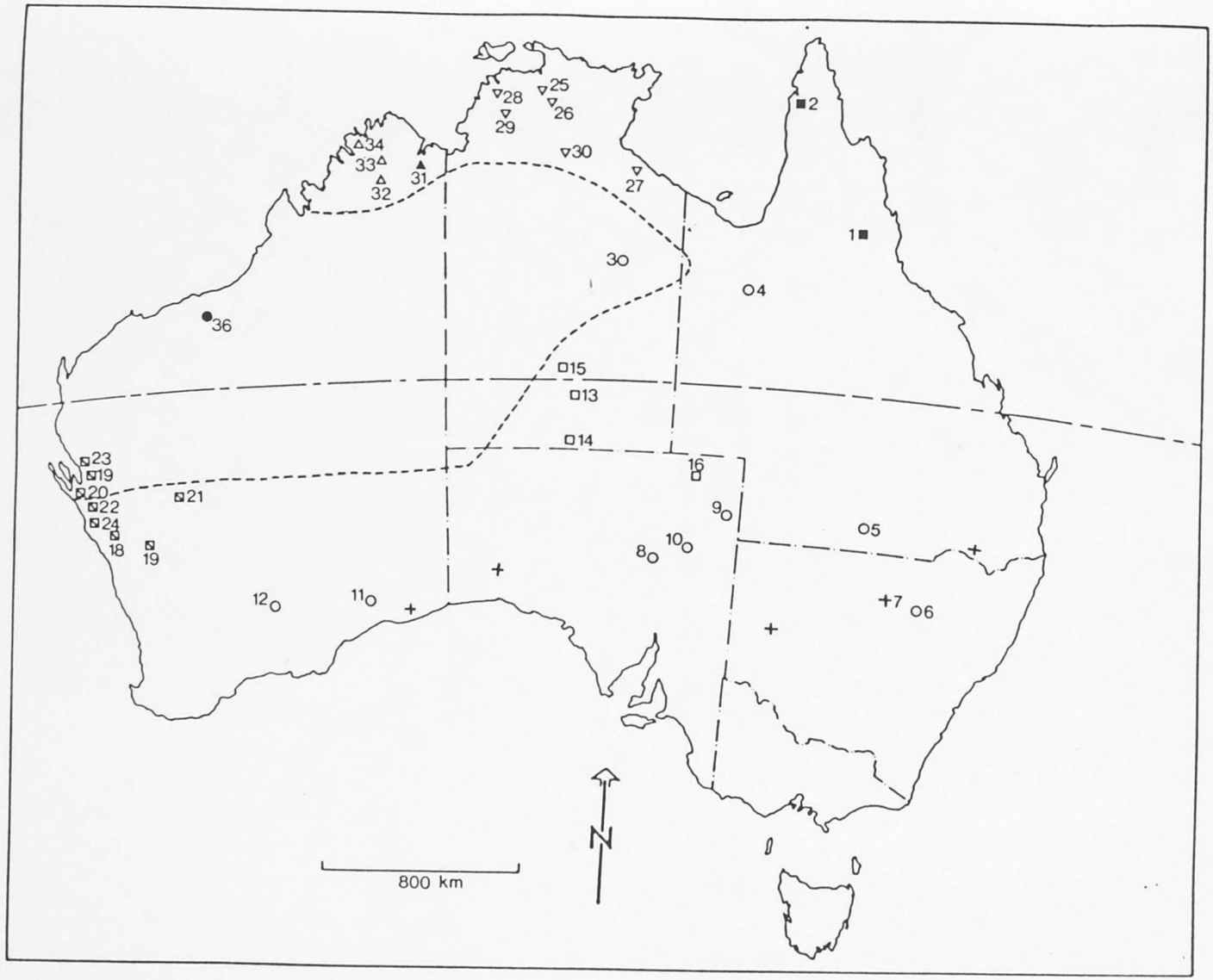

800 km
N

Figure 3.3 Silver stained metaphase spread of a diploid CA6 individual from the MacDonnell Ranges. Note that despite the absence of a secondary constriction the NOR remains on chromosome 6.

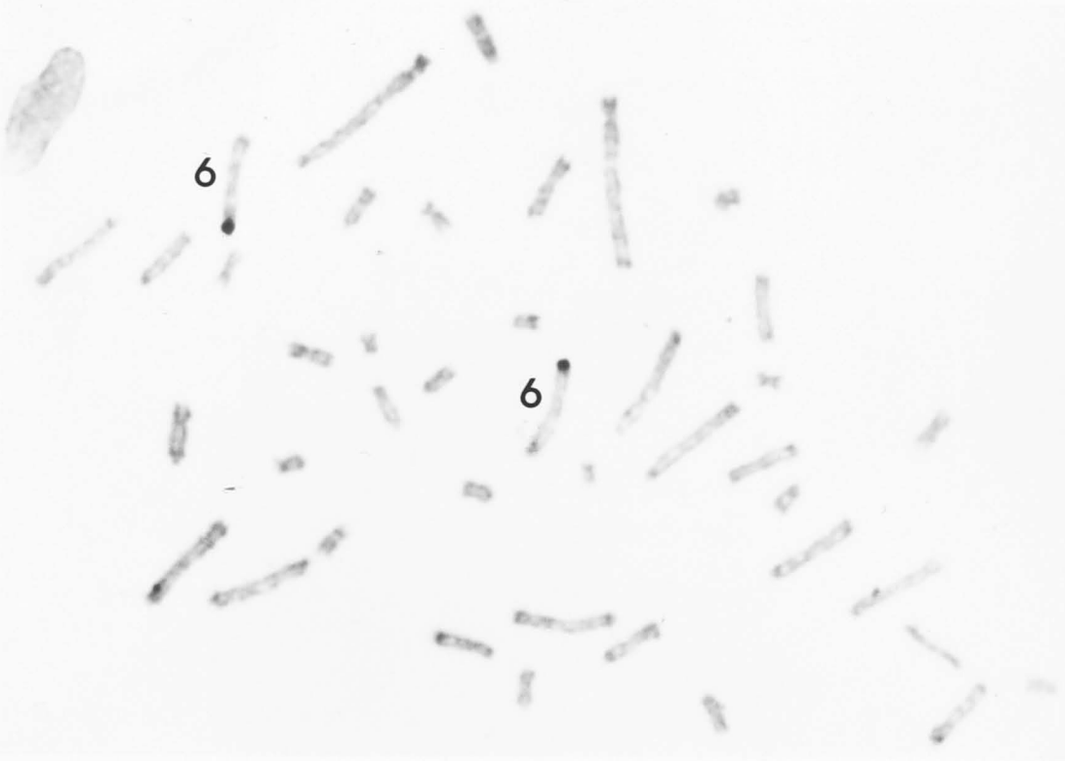

6
6

Figure 3.4 The geographic distribution of the various SM6 karyomorphs in the western two-thirds of Australia. State borders and the tropic of Capricorn are shown. The numbers refer to table 3.2. Note the disjunct distribution of the SM6-3 cytotype (SM6-3a, SM6-3b).

Symbols :

△ SM6-1a

▲ SM6-1b

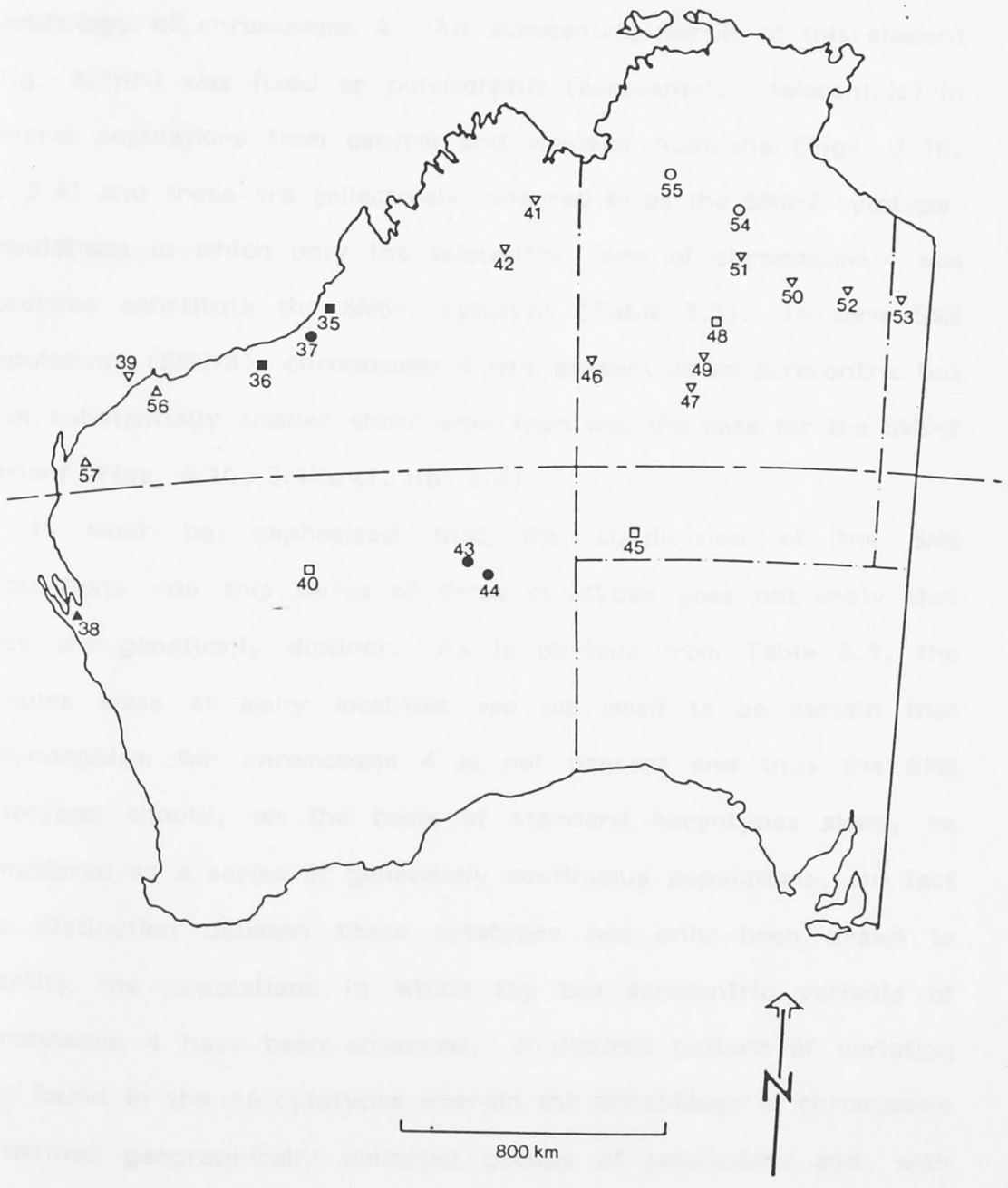
□ SM6-2a

▽ SM6-2b

○ SM6-3a

■ SM6-3b

● SM6-1; no c-banding


55
41
54
42
51
50
52
53
35
48
37
39
36
46
49
56
47
57
45
43
40
44
38
800 km
N

(cf. Figs 3.1Hf and g). The SM6 variant was fixed in a series of populations from central and western regions (Figs. 3.1E,F, G, 3.2, 3.4) and these samples can be further subdivided on the basis of the morphology of chromosome 4. An acrocentric morph of this element (Fig. 3.1Hb) was fixed or polymorphic (acrocentric: telocentric) in several populations from central and western Australia (Figs. 3.1E, F, 3.4) and these are collectively referred to as the SM6-2 cytotype. Populations in which only the telocentric form of chromosome 4 was observed constitute the SM6-1 cytotype (Table 3.3). In some SM6 populations (SM6-3), chromosome 4 was present as an acrocentric but with substantially smaller short arms than was the case for the SM6-2 variant (Figs. 3.1G, 3.1Hc cf. Hb, 3.4).

It must be emphasised that the subdivision of the SM6 populations into this series of three cytotypes does not imply that they are genetically distinct. As is obvious from Table 3.3, the samples sizes at many localities are too small to be certain that polymorphism for chromosome 4 is not present and thus the SM6 cytotypes should, on the basis of standard karyotypes alone, be considered as a series of genetically continuous populations. In fact the distinction between these cytotypes has only been drawn to identify the populations in which the two acrocentric variants of chromosome 4 have been observed. A distinct pattern of variation was found in the A6 cytotypes wherein the morphology of chromosome 6 defined geographically delimited groups of populations and, with respect to the presence or absence of a secondary constriction, they were characterised by alternative fixed character states.

The dichotomy between the A6 and SM6 cytotypes is quite fundamental and they may represent distinct biological species.

Despite their broadly overlapping distributions (cf. Figs. 3.2 and 3.4) they have only been observed in sympatry once, and at this locality they evidently maintained separate gene pools. At Bamboo Ck (locality number 36, Fig. 3.4) H. binoei (SM6) and H. spelea (A6 with secondary constriction) were sympatric and these species are morphologically and ecologically divergent (Kluge 1963). Furthermore, none of the 266 diploid H. binoei karyotyped were heterozygous for the chromosome 6 variants that distinguish that A6 and SM6 cytotypes.

In addition to the variants of chromosomes 4 and 6 which were used to define the cytotypes described above, two other polymorphisms were identified at this levels of resolution. In three of the A6-2 populations from northern Australia (locality numbers 27, 29, 30, Fig. 3.2) polymorphism for a telocentric:acrocentric combination of chromosome 1 was observed (Fig. 3.1C) and in one of the one A6 samples from south Australia (locality number 16, Fig. 3.2) there was a polymorphic variant of chromosome 4 with minute arms (Fig. 3.1D). For each of these rearrangements, and the variants of chromosome 4 and 6 described above, the C-banding analysis demonstrated that they were not due to amplification or deletion of heterochromatin (Fig. 3.6a, e, d, 3.7d).

Diploid Heteronotia - C-band patterns

The application of the C-banding technique to Heteronotia has revealed a complex pattern of fine proximal, interstitial and distal bands. With the exception of the samples from Cape York Peninsular (CYA6) and Wyndham (WYA6) which lacked detectable interstitial bands (Fig. 3.6), it proved possible to consistently identify

chromosomes 1 to 6 on the basis of both their size and their C-banding pattern (Fig. 3.5). The study of C-band variation in Heteronotia was therefore restricted to these six pairs of chromosomes.

The analysis revealed marked discontinuities between geographic regions in the precise distribution of interstitial C-bands on chromosomes 1 to 6 and on this basis, the karyomorphs illustrated diagrammatically in Figure 3.8 were recognised. Four such karyomorphs were defined in the A6 populations and a further three were recognised within the A6-2 samples (Figs. 3.2, 3.6, 3.8). Within the SM6 group a further five karyomorphs were recognised on the basis of their C-banding patterns and structural rearrangements of chromosome 4 (Figs. 3,4, 3.7, 3.8). The SM6 karyomorphs displayed more continuity in their patterns of variation than did the A6 and A6-2 karyomorphs (Figs. 3.7, 3.8) which supports the notion that they represent a complex of closely related populations (see above).

The CA6 karyomorph is defined by the polytypic morphs of chromosomes 2 to 6 shown in Figure 3.8 and in the western samples of this karyomorph (Fig. 3.2) a variant of chromosome 4 with a strong procentric C-band (Fig. 3.6g) was found to be fixed or polymorphic. An identical morph of chromosome 4 (Fig. 3.7c) was also fixed or polymorphic in some populations of the SM6-1b and SM6-2 karyomorphs (Fig. 3.4).

The C-banding analysis clearly substantiates the dichotomy between the A6 and A6-2 cytotypes on one hand and the SM6 group on the other (cf. Figs. 3.6 and 3.7 and see Fig. 3.8). Thus, in the karyomorphs where chromosomes 3 and 5 had interstitial bands, the

Figure 3.5 Full C-banded cell from a central Australian A6 diploid individual to show the nature of the size and C-band patterns of chromosomes 1 to 6.

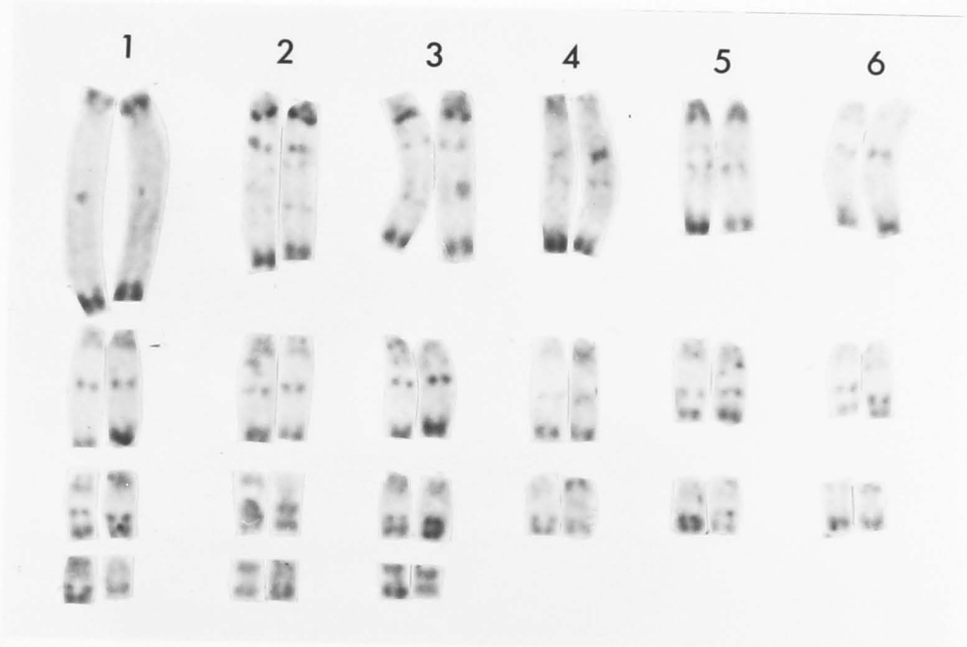

1
2
3
4
5
6

Figure 3.6 Composite figure showing polytypic (Pt) and polymorphic (Pm) C-band variants of chromosomes 1 to 6 in the A6 and A6-2 cytotypes of bisexual Heteronotia. Polytypic variants characterise four geographically determined karyomorphs within the A6 cytotype, namely, the CA6, CYA6, EA6 and SPELEA. A further three such karyomorphs were defined in the A6-2 cytotype. These are referred to as NA6, KA6 and WYA6.

a-l). Polymorphic variants discussed in text. Note the prevalence of polymorphism in chromosome 4.

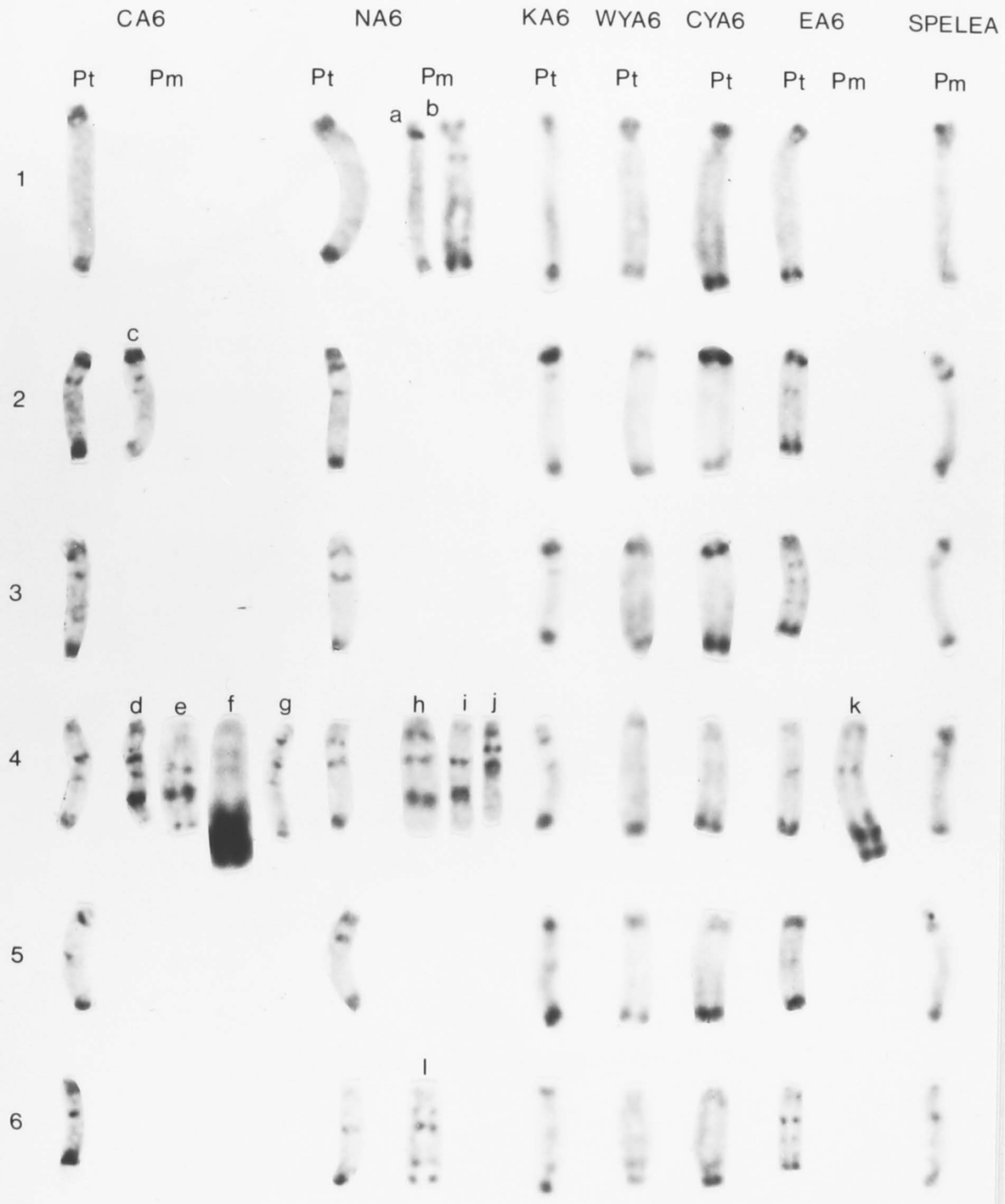
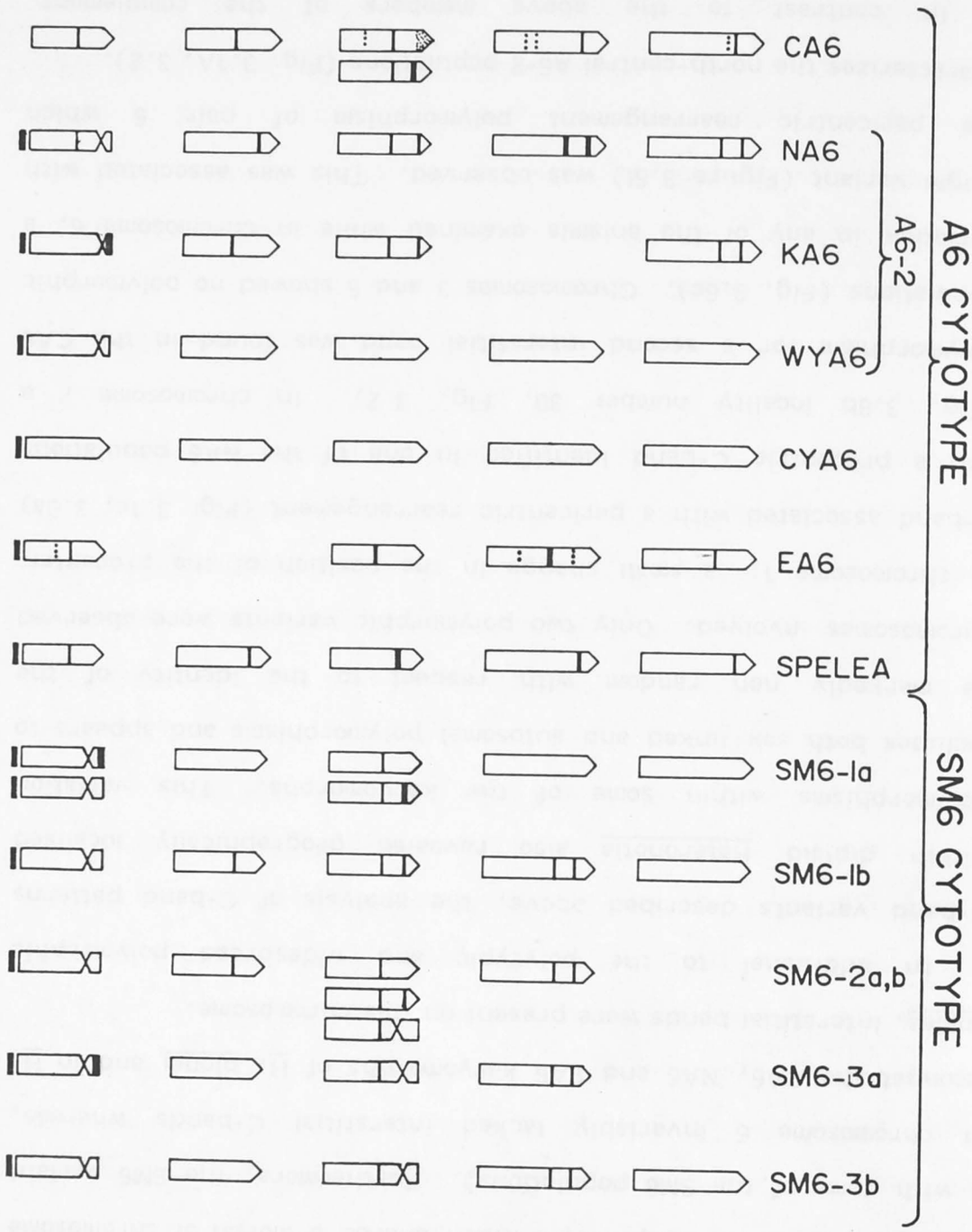

CA6
NA6
KA6
WYA6
CYA6
EA6
SPELEA
Pt
Pm
Pt
Pm
Pt
Pt
Pt
Pt
Pm
Pm
1
2
3
4
5
6
a
b
c
d
e
f
g
h
i
j
k
l

Figure 3.8 Combinations of polytypic variants of chromosomes 2 to 6 that define the karyomorphs within the A6 and SM6 groups. For clarity, only the variable interstitial bands are shown and blank spaces indicate that a morph identical to that in the CA6 karyomorph is present. Some important polymorphic markers of chromosome 4 that were identified in the CA6 karyomorph and some of the SM6 karyomorphs are also shown.


CA6
NA6
KA6
WYA6
CYA6
EA6
SPELEA
SM6-1a
SM6-1b
SM6-2a,b
SM6-3a
SM6-3b
A6-2
A6 CYTOTYPE
SM6 CYTOTYPE

precise position of these bands differed between these groups (excluding the NA6 karyomorph which shared a morph of chromosome 3 with some of the SM6 populations). Furthermore, the SM6 variant of chromosome 6 invariably lacked interstitial C-bands whereas, amongst the CA6, NA6 and EA6 karyomorphs of H. bionei and in H. spelea, interstitial bands were present on this chromosome.

In additional to the polytypic and widespread polymorphic C-band variants described above, the analysis of C-band patterns within diploid Heteronotia also revealed geographically localised polymorphisms within some of the karyomorphs. This variation includes both sex linked and autosomal polymorphisms and appears to be markedly non random with respect to the identity of the chromosomes involved. Only two polymorphic variants were observed in chromosome 1; a small change in the position of the procentric C-band associated with a pericentric rearrangement (Fig. 3.1c, 3.6a) and a procentric C-band identified in one of the NA6 populations (Fig. 3.6b locality number 30, Fig. 3.2). In chromosome 2 a polymorphism for a second interstitial band was found in the CA6 populations (Fig. 3.6c). Chromosomes 3 and 5 showed no polymorphic variation in any of the aniamls examined while in chromosome 6, a single variant (Figure 3.6l) was observed. This was associated with the pericentric rearrangement polymorphism of pair 6 which characterizes the north-central A6-2 populations (Fig. 3.1A, 3.2).

In contrast to the above members of the complement, polymorphism was extremely common in chromosome 4. A variant of chromosome 4 (Fig. 3.6d) which occurs in the MacDonnell Range CA6 populations (locality 13, Fig. 3.2) is clearly sex linked. This morph which differs in the position of the terminal C-band was heterozygous in all 18 females but was absent from the 20 males analysed and thus

represents a ZW sex chromosome heteromorphism. Several other variants of chromosome 4 in which the position of the terminal C-band has been modified have been identified in both the A6 and SM6 groups (Fig. 3.9, Table 3.4). In each case the modified chromosome was heterozygous in females and absent in males which is strongly indicative of additional ZW sex chromosome heteromorphisms, although the sample sizes per locality are generally too small to provide definitive proof of this. If all of these morphs are in fact W chromosomes, then it is of particular interest that in each of the karyomorphs in which they have been identified, they only occur in some populations (Table 3.4).

A further four morphs of chromosome 4 which carried large terminal heterochromatin amplifications were identified in both males and females (Figs. 3.6f,k, 3.7a,b). In each case the interstitial bands corresponded to those found in chromosome 4 of the appropriate karyomorph which may indicate that each amplification was an independant event.

The prevalence of chromosome polymorphism in chromosome 4 of H. binoei is principally due to the large number of distinct W chromosomes (Fig. 3.9). However, it should be noted that of the eight types of W chromosome so far identified two may have been generated by recombination. As shown in Figure 3.9, the W_c morph is differentiated from the W_d form by the presence of a strong procentric C-band in the former. This variant could have arisen through recombination between the W_d morph and a Z chromosome with this band. The procentric band of the W_g morph may be the result of a similar process.

Figure 3.9 Composite figure showing the eight morphs of the W chromosome (pair 4) identified within H. binoei. The W chromosome is on the left and the corresponding Z chromosome is on the right. The populations in which these variants were identified are listed in table 3.4. Note that in some cases, only minor differences in C-banding distinguish these variants. For example the h morph (from an SM6-3 population) only differs from the f morph (from SM6-2 populations) by the absence of C-band heterochromatin on the distal telomere of the former.

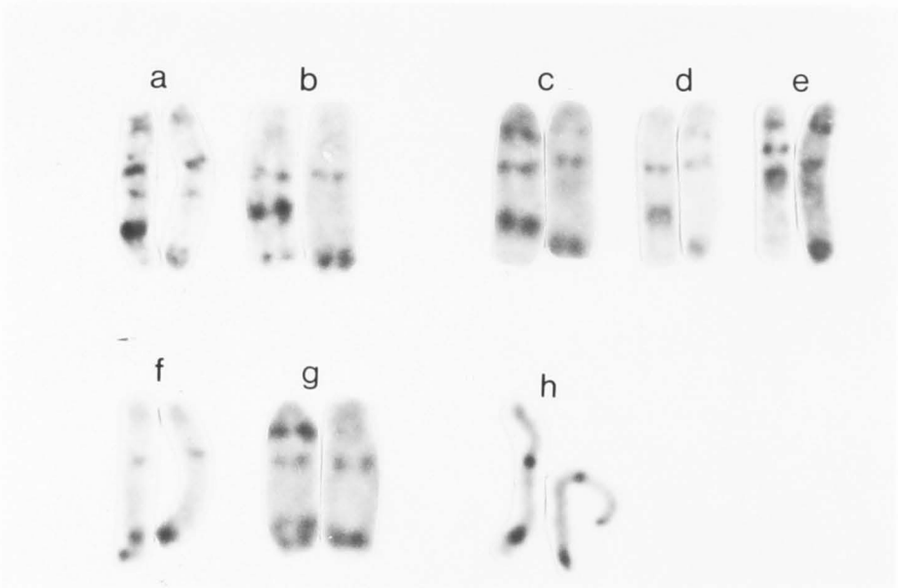

a
b
c
d
e
f
g
h

Table 3.4

W chromosome variation in Heteronotia. The morph of the W chromosome that is present refers to the variants shown in figure 3.9. The exception is for H. sp. nov. in which a large structural rearrangement of chromosome 6 to appears to define a W chromosome (Fig. 3.10). Note that females sampled from many populations did not have a cytologically differentiated W chromosome. No males were found to have these variants.

TABLE 3.4

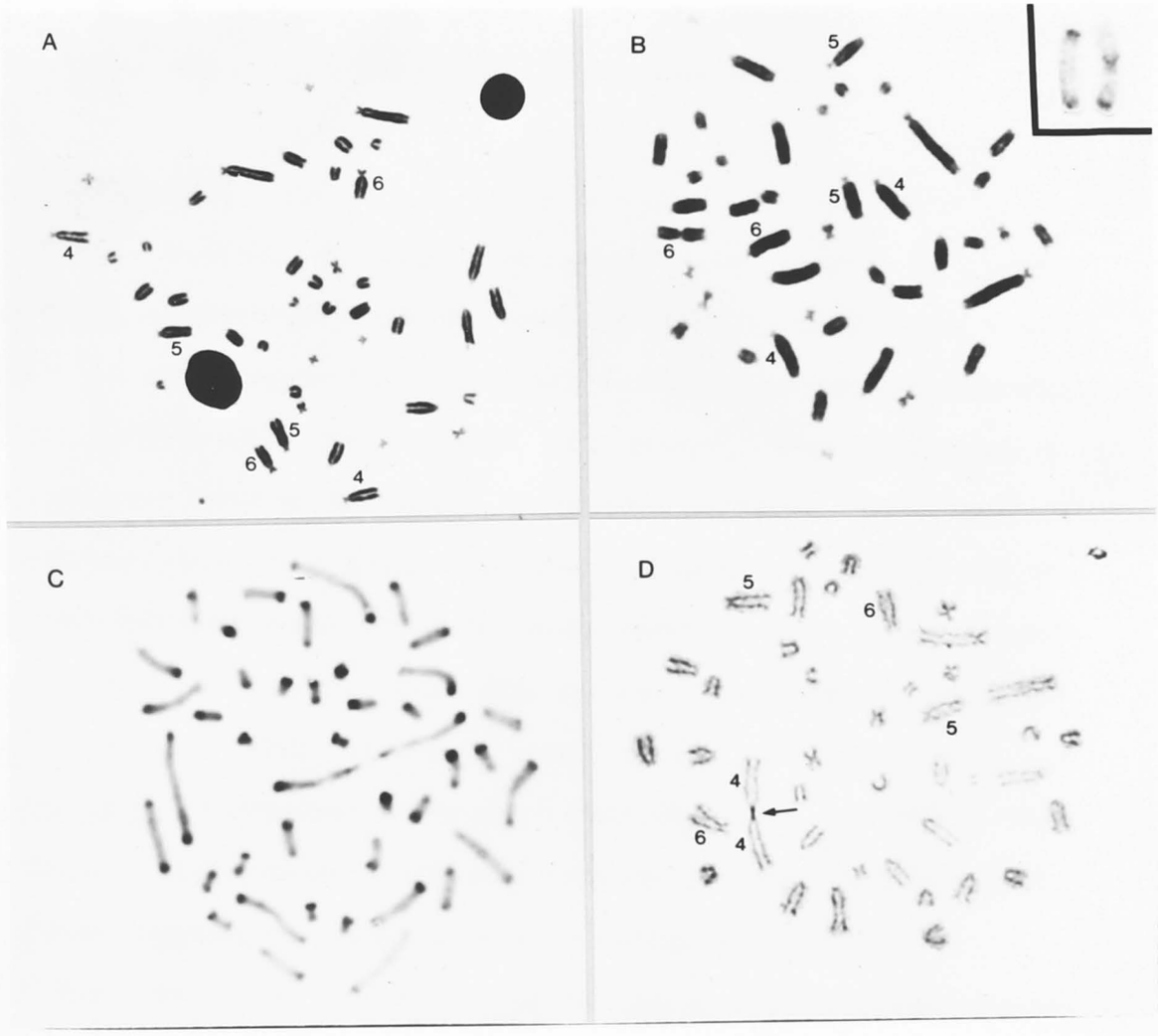
LOCALITY	KARYOMORPH	Number of females C-banded	W chromosome present
13. MacDonnell Range	CA6	18	W_a
14. Kulgera	CA6	2	none
15. Native Gap	CA6	1	none
16. Cordillo Downs	CA6	1	W_b
26. Baroalba	NA6	2	W_c
27. Bing Bong	NA6	2	W_d
29. Hayes Ck.	NA6	1	W_c
30. Mataranka	NA6	2	W_c, W_e
28. Darwin	NA6	1	none
48. Wauchope	SM6-2a	1	W_f
41. Turkey Ck.	SM6-2b	2	W_g
42. Halls Ck.	SM6-2b	1	none
39. Enderby Isl.	SM6-2b	1	none
45. Ayers Rock	SM6-2a	1	none
51. Renner Springs	SM6-2b	1	none
53. Camooweal	SM6-2b	1	none
54. Daly Waters	SM6-3a	1	W_h
55. Willeroo	SM6-3a	1	W_h
56. Karratha	SM6-3b	1	none
57. Nanutarra	SM6-3b	1	none
13. Ross River	SP. NOV. (A6-3)	1	4♀♀ all meta: acro-pair 6
13. Undoolya	SP. NOV. (SM6-4)	5	none
13. Bond Springs	SP. NOV. (SM6-4)	1	none

Nevertheless, it is clear that the highly variable nature of chromosome 4 relative to the other members of the karyotype is due to the large number of C-band changes, which possibly reflect small paracentric inversions and are associated with a sex determing region located distally on chromosome 4. The significance and possible causes of highly variable W chromosomes are the subject of chapter 5 which reports another more dramatic example of this phenomenon in Gehyra purpurascens.

In addition to the chromosome variation described above, a karyotypically distinct form of Heteronotia coexists with the CA6 karyomorph of H. binoei in the MacDonnell Ranges of central Australia (locality 13, Fig. 3.2). These individuals are morphologically similar to, but not identical with H. spelea and are referred to here as H. sp. nov. pending formal taxonomic description (Moritz, Rowell and Bugledich, unpublished). As is the case for other Heteronotia, these are 2n = 2x = 42 with a predominantly telocentric karyotype (Fig. 3.10A, B). However, H. sp. nov. is unique in having the NOR on the short arms of chromosome 4 (Fig. 3.10D) and all individuals examined (see Table 3.3) lacked interstitial C-bands (Fig. 3.10C).

Within the restricted distribution of this species, two distinct karyotypes were found. In populations from the central and western MacDonnell Ranges, chromosome 6 was invariably telocentric (Fig. 3.10A) whereas in the sample of eight individuals from Ross River, which is approximately 40 km to the east of the other samples, pair 6 was polymorphic for a telocentric:metacentric combination (Fig. 3.10B). This polymorphism may also represent a ZW sex chromosome polymorphism since the four females examined from this population were heterozygous for the metacentric chromosome which was absent

Figure 3.10 (A-D) The karyotype of H. sp. nov. from the MacDonnell Ranges of central Australia. A) Standard karyotype of H. sp. nov. from several localities in the central and northern MacDonnell Ranges. B) Standard karyotype of H. sp. nov. from the Ross River locality. Note the telocentric nature of one homologue of chromosome 6 and that in this female the other homologue is metacentric. This rearrangement appears to represent another ZW system with the W being metacentric. Inset - C-banding of the ZW chromosome pair. Note the centric heterochromatin of the metacentric W (right). C) A C-banded cell of H. sp. nov. which shows the absence of interstitial C-band material. D) A silver stained cell of H. sp. nov. which shows that, in contrast to other Heteronotia (cf. fig. 3.3), the active NOR is located on the short arms of chromosome 4. In this cell the NOR's of the two homologues are associated.


A
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4
C
D
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6
4

in the four males (Table 3.4). This observation is of particular interest since it suggests that the location of the primary sex determining region differs between H. binoei and H. sp. nov., although it must be recognised that in the absence of interstitial C-bands in the latter species, it is extremely difficult to unambiguously identify the specific chromosome pairs.

This analysis of karyotypic variation in diploid Heteronotia presents a picture of quite restricted variation in the standard format with the only observed variants being small pericentric rearrangments that vary within and between populations. The application of chromosome banding techniques to reptiles generally, and lizards in particular, has been quite restricted (Bickham, in press) and no studies to date have employed these techniques to reveal cryptic variation in lizards in which the standard karyotype is relatively conservative. This approach does appear profitable since in Heteronotia substantial variation, involving both sex-linked and autosomal local polymorphism and regional polytypic differentiation, has been revealed by the use of the C-banding technique.

The main value of the analysis is that it enables subdivision of the widespread A6 and SM6 cytotypes into geographically delimited karyomorphs and thereby provides useful genetic markers to resolve the origin and evolution of the triploid parthenogenetic biotype of H. binoei.

(ii) Triploid H. binoei (2n = 3X = 63)

A total of 189 triploid females from central and western Australia have been karyotyped and of these, 71 have been C-banded. No triploid males have been identified in H. binoei. The results of this analysis, summarised in Table 3.5, have revealed three distinct classes of clones: (1) widespread clones that have representatives in both central and western Australia, (ii) geographically restricted clones and (iii) chromosomally derived clones.

Widespread clones

On the basis of standard karyotypes, three clones (A-1, B-1 and C-1) have been identified. All of these are heteromorphic for the pericentric rearrangement on chromosome 6 that distinguishes the A6 and SM6 bisexual cytotypes and clone C-1 is also heterozygous for the acrocentric chromosome 4 morph that defines the SM6-2 cytotype. Specifically, clone A-1 is characterised by one submetacentric chromosome 6 (Fig. 3.11A), clone B-1 has two such chromosomes (Fig. 3.11B) while clone C-1 also has two but is also heteromorphic for chromosome 4 (Fig. 3.11C). In each of these clones, secondary constrictions, when present, were restricted to the submetacentric chromosome 6. All of these clones have remarkably widespread distributions (Fig. 3.12) and the parthenogenetic biotype of H. binoei as a whole, has the broadest distribution of any known continental subsexual vertebrate.

The C-banding technique was applied to these clones to increase the resolution of the chromosome analysis and a complete C-banded triploid cell of H. binoei (clone A-1b) is shown in Figure 3.13. As was the case for the diploids, detailed analysis was restricted to

Table 3.5

Localities from which triploid H. binoei were karyotyped and the cytogenetic clones identified in each sample. Note that the number of individuals C-banded from any one locality is very low. The localities at which both diploids and triploids are identified and at some sites, (1, 28, 38) single tetraploid females were discovered. The numbers (1-54) refer to the points on figure 3.12.

TABLE 3.5

LOCALITY	SAMPLE SIZE STANDARD KARYOTYPES	SAMPLE SIZE C-BANDING	CLONES IDENTIFIED
1. Ross River Stn*+. N.T.	16	2	A-1a, A-1d
2. Undoolya Stn*. N.T.	10	4	A-1b
3. Deep Well Stn. N.T.	2	-	A-1, C-1
4. Kulgera* N.T.	10	5	A-1a, A-1c
5. Finke N.T.	2	2	A-1a
6. Orange Ck. Stn. N.T.	10	4	A-1a, C-1b
7. Lilla Ck. Stn. N.T.	5	-	A-1
8. Horseshoe Bend Stn N.T.	8	2	A-1a, C-1b
9. Umbeara Stn. N.T.	4	2	A-1a
10. Ayers Range N.T.	2	-	A-1
11. Alice Springs* N.T.	1	1	A-1a
12. Aileron* N.T.	4	2	B-5, B-1
13. Tea Tree* N.T.	2	1	B-1a
14. Old Andado Stn N.T.	6	2	B-1a, C-1b
15. Mt. Ebenezer N.T.	4	1	B-1a, C-1
16. Curtin Springs N.T.	3	1	B-1a
17. Ayers Rock* N.T.	9	3	B-1a
18. Docker River N.T.	2	1	B-1a
19. Rawlinson Ra. W.A.	1	-	A-1
20. Giles W.A.	3	1	B-1a
21. Warrakurna W.A.	1	-	B-1
22. Wingelinna W.A.	3	3	B-1a, C-1b
23. Warburton W.A.	5	1	C-1, C-3
24. McDouall Peak Stn. S.A.	1	-	C-1
25. Wirraminna Stn. S.A.	4	1	C-1b
26. Mt. Willoughby Stn. S.A.	2	-	A-1, C-1
27. Welbourne Hill Stn. S.A.	1	-	A-1
28. Randalls Siding*+ W.A.	1	1	A-1a
29. Broad Arrow W.A.	1	1	A-1c
30. 90 km Nth Koolyanobbin W.A.	1	1	A-1c
31. Victory Rock W.A.	1	-	A-1
32. Johnstone Rocks W.A.	3	2	A-1a, A-1b
33. Hospital Rocks W.A.	1	1	A-1b
34. Emu Fence (30° 23'x 118° 31') W.A.	3	1	A-1c
35. 60 km Nth Agnew W.A.	6	1	A-1b, A-3
36. Laverton Downs Stn W.A.	6	1	A-1b
37. Cowarna Stn. W.A.	2	2	A-1c
38. Rawlinna*+ W.A.	1	1	A-1c
39. Kijanding Rock W.A.	1	-	A-1
40. Leonora	1	-	B-1
41. 70 km SW Wongawol Stn W.A.	1	1	A-1b
42. Carnegie Stn. W.A.	5	3	B-1a, C-1, C-2
43. Glenayle Stn.* W.A.	3	2	C-1a
44. Milbillillie Stn. W.A.	5	1	A-1a, C-1, B-1
45. Doolgunna Stn. W.A.	5	4	A-1b, B-1b
46 5 km Sth Cue W.A.	2	-	A-2
47. Tallering Stn. W.A.	2	-	A-1
48. Thundelarra* Stn. W.A.	4	1	A-2, A-3
49. Tarmoola Stn. W.A.	3	3	A-3, B-1a
50. Booloogooroo Stn. W.A.	4	3	B-3
51. 140 Km Sth. Carnarvon W.A.	1	-	A-1
52. Quobba W.A.	1	-	A-1
53. Nanutarra* Stn W.A.	1	1	D
54. Roy Hill Stn. W.A.	3	2	B-2, B-4

* sympatric with diploid H. binoei (see Table 3.3)

+ tetraploids identified.

Figure 3.11 (a-d) Standard karyotypes of the three widespread triploid clones (a to c) of H. binoei and a tetraploid female (d). (a) Clone A-1 with a single submetacentric 6 which has a distal secondary constriction. (b) Clone B-1 with two submetacentric 6's, both with distal secondary constrictions. (c) Clone C-1 which, in addition to two submetacentric 6's, is heteromorphic for short arms on chromosome 4. (d) Karyotype of a tetraploid with a single submetacentric 6.

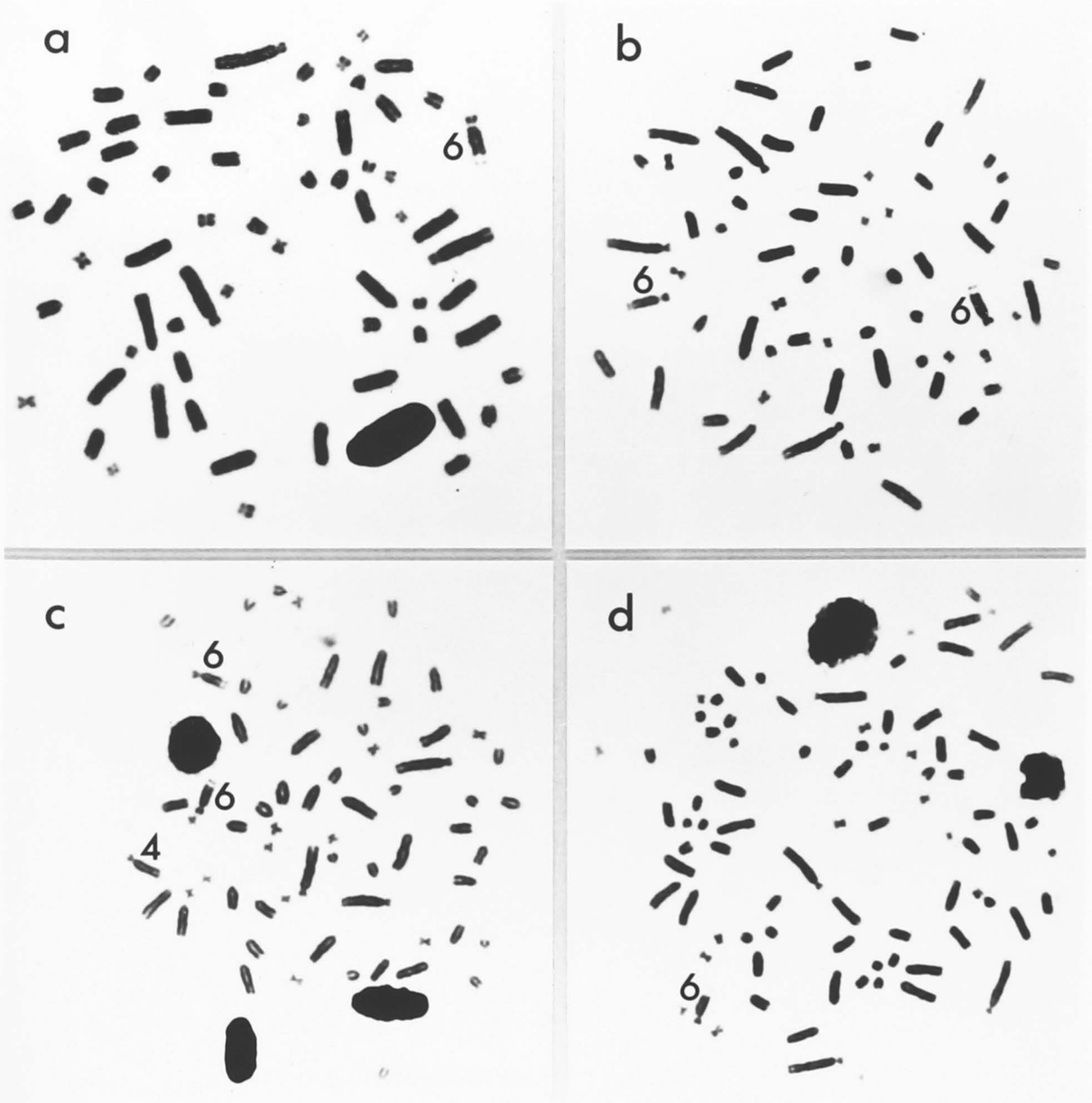

a
6
b
6
6
c
6
6
4
d
6

Figure 3.12 The geographic distribution of the various triploid clones of H. binoei. The area shown covers the south-central and south western region of the continent; state boundaries and the tropic of Capricorn are shown. The numbers refer to table 3.4.

Symbols :

□(a-d)	clone A-1 (a-d)	
△(a-b)	clone B-1 (a-b)	WIDESPREAD
▽(a-b)	clone C-1 (a-b)	CLONES
○	clone A-2	
■	clone A-3	RESTRICTED
▲	clone B-2	CLONES
●	clone B-3	
+	clone D	

C-2, C-3, B-4, B-5 refer to chromosomally derived clones.

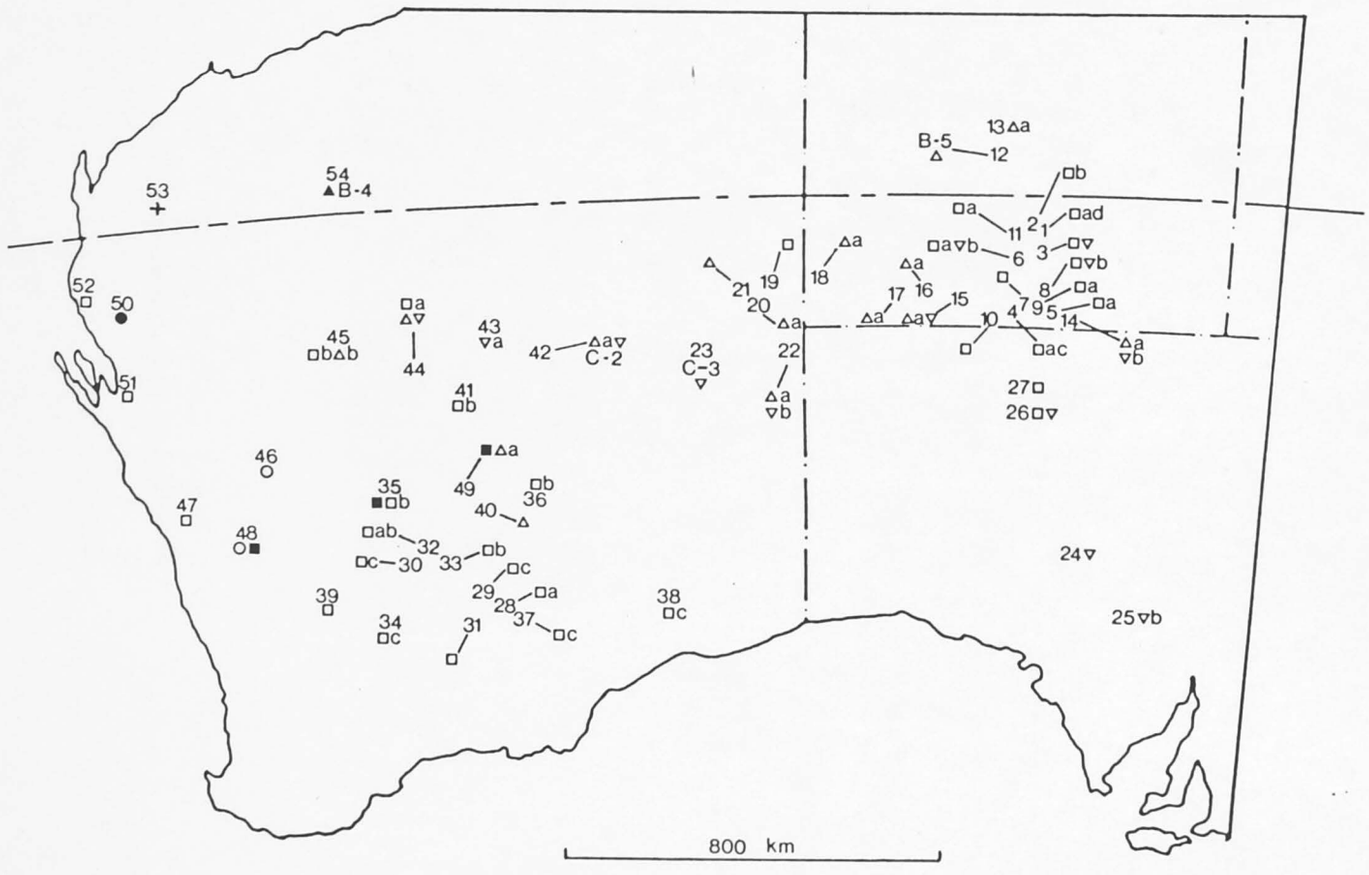
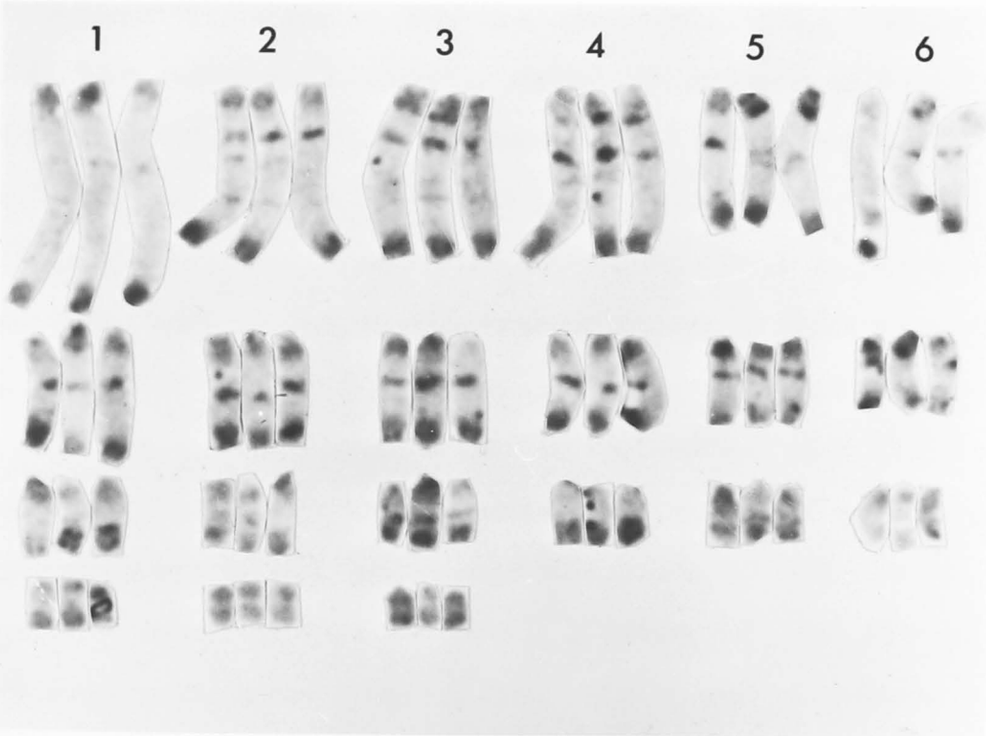

B-5
13△a
12
54
▲B-4
53
+
□b
□a
11
2
1
□ad
□a▽b
6
3
□▽
□▽b
8
□a
□a
14
△a
▽b
52
□
50
●
51
□
45
□b△b
44
□a
△▽
43
▽a
42
△a▽
C-2
23
C-3
▽
22
△a
▽b
41
□b
21
19
18
△a
20
△a
17
16
△a
△a▽
15
10
4
7
9
5
□ac
27□
26□▽
46
○
47
□
48
○■
35
■□b
49
■△a
□b
36
40
△
□ab
32
□b
□c
30
33
□c
39
□
34
□c
29
28
□a
37
31
□
□c
38
□c
24▽
25▽b
800 km

Figure 3.13 Full C-banded cell of a triploid H. binoei.


1
2
3
4
5
6

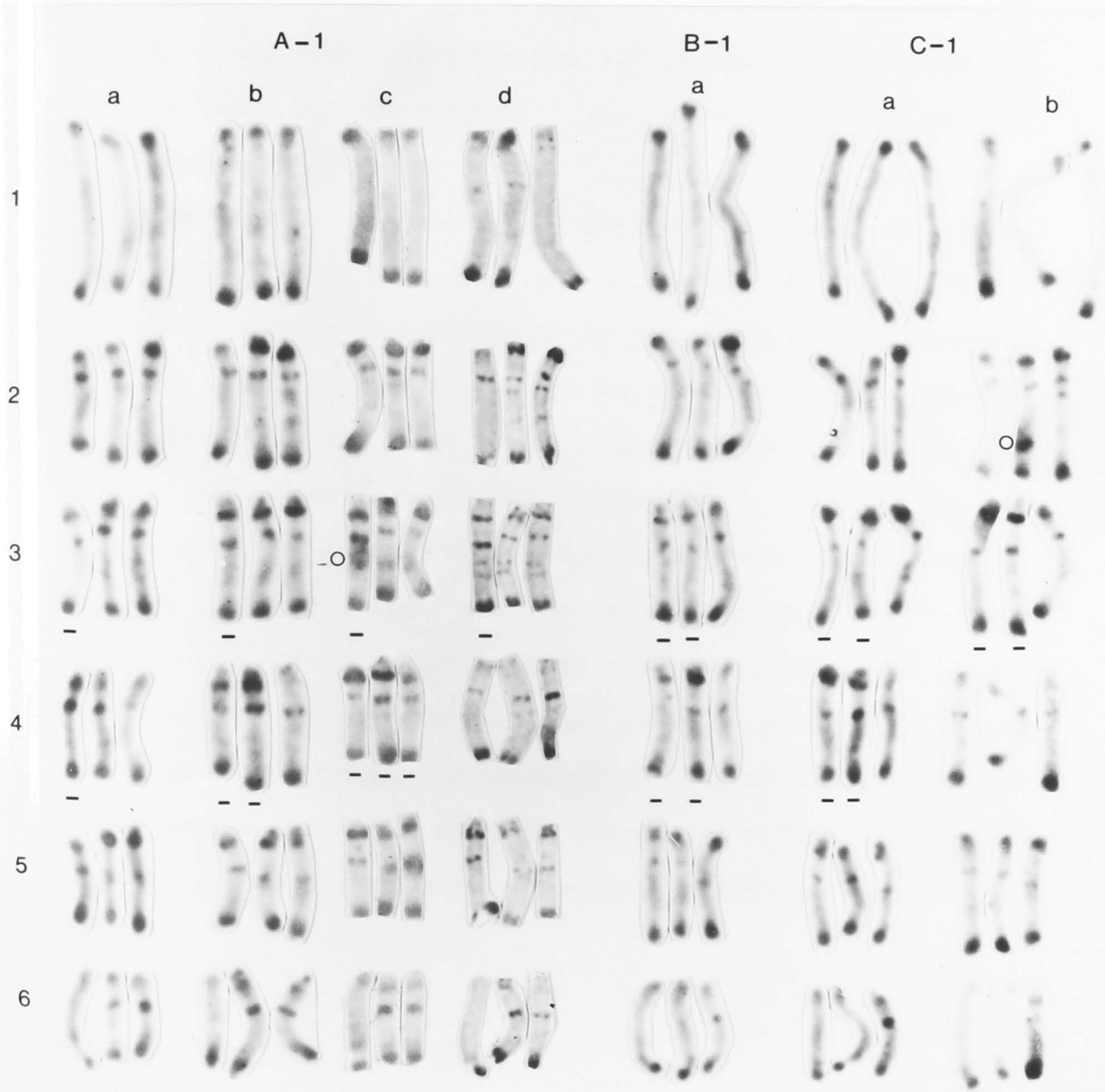
chromosomes 1 to 6 as only these could be unambiguously identified. The more distal of the two interstitial bands on chromosome 2 could not be reliably scored in all triploids and is therefore excluded from the analysis.

C-banding made it clear that clone A-1 as defined by conventional giemsa-staining was, in reality, a group of four clones (A-1a to A-1d) that were distinguishable by the variants of chromosome 4 present in any one clone (Fig. 3.14). Thus, clone A-1d was homozygous for the variant of chromosome 4 lacking a procentric C-band, clone A-1a had one member with this band, clone A-1b had two and clone A-1c was homozgyous for this variant (Fig. 3.14). These four clones were distinguished from the various B-1 and C-1 clones by the heteromorphisms present in chromosomes 3 and 6. In the A-1 clones, there was one representative of the chromosome 3 morph characteristic of the SM6 and NA6 karyomorphs (see Fig. 3.8) and for chromosome 6 there was one unbanded submetacentric 6 and two banded telocentric 6's (Fig. 3.14). As would be predicted from the standard karyotypes, the B-1 and C-1 clones were the mirror image of this. That is, for chromosome 3 they had two of the NA6/SM6 variants and for chromosome 6 they had two submetacentrics and a single telocentric with an interstitial band (Fig. 3.14). Within the C-1 standard karyotypic clone there were two clones that could be distinguished by the C-band pattern of chromosome 4. Clone C-1a had two chromosomes with a strong procentric C-band whereas clone C-1b had none (Fig. 3.14).

The absence of secondary constrictions on the telocentric representatives of chromosome 6 in these clones prompted an analysis of silver staining patterns to determine location of active NOR's in the

Figure 3.14 Composite figure showing the C-band variation observed both within and between the geographically widespread triploid clones of H. binoei. When the C-band pattern of the A-1 clones are compared to those of the B-1 and C-1 clones the heteromorphisms of chromosomes 3, 5 and 6 are seen to conform to their respective number of A6 and SM6 genomes. The underlined representatives of chromosome 3 have a lower interstitial band which is characteristic of some SM6 karyomorphs (and the NA6 karyomorph - see figure 3.8). Within the A-1 clone as defined by standard karyotype analysis, four clones which differed in the number of members of the chromosome 4 triad bearing a strong proximal C-band (underlined) could be distinguished. On the same basis, two clones could be resolved within clone C-1.

o = overlap


A-1
B-1
C-1
a
b
c
d
a
a
b
1
2
3
4
5
6

triploid H. binoei. The analysis of 21 individuals from clone A-1 showed that active NOR's were present distally on submetacentric and telocentric morphs of chromosome 6 (Fig. 3.15) despite the absence of a secondary constriction in the latter. Thus on the basis of both C-banding and NOR characteristics the telocentric chromosome 6's were equivalent to those observed in the CA6 diploid karyomorph (see Figs. 3.3 and 3.6).

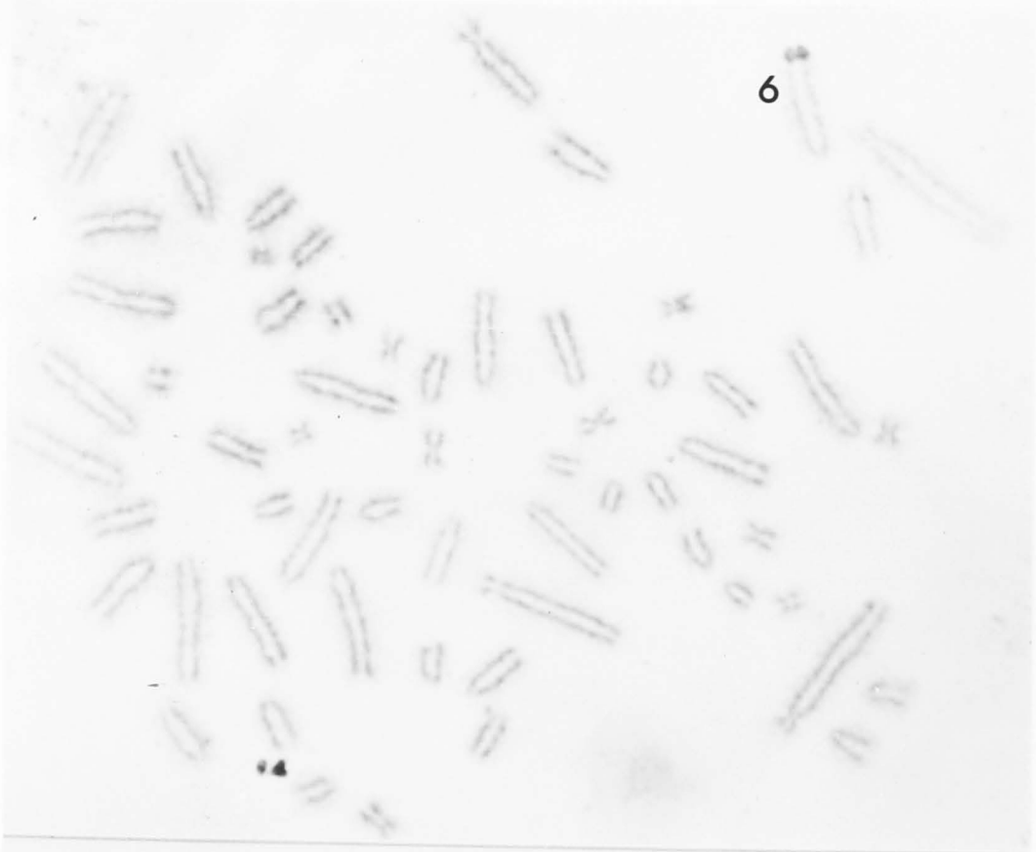
Within a triploid individual, there may be one or two active NOR's (Fig. 3.15) with only the occasional cell having three. However an analysis of the physical distribution of 18 + 28S ribosomal genes by in situ hybridisation of a probe derived from Drosophila has demonstrated that the three sites of ribosomal genes expected in a triploid are indeed present (Fig. 3.16A, B). Comparison of this finding with the silver staining analysis of active NOR's therefore provides admittedly crude evidence for dosage compensation since very few cells (5 out of 56) analysed had three active NOR's. This result parallels the situation in diploid/tetraploid complexes of fishes and frogs where dosage compensation is achieved through reduced transcriptional activity of ribosomal cistrons in addition to a higher turnover rate of the transcribed molecules (Leipoldt and Schmidtke, 1982).

Geographically restricted clones

The analysis of standard karyotypes from west Australian H. binoei revealed a series of a further four clones that, in addition to heteromorphisms for chromosome 6, carried a single acrocentric chromosome 2 with minute short arms as well as two of the standard telocentric chromosome 2's (Fig. 3.17 A-D). Each of these clones

Figure 3.15

Silver staining patterns of triploid and tetraploid H. binoei. Top: Full cell from a triploid clone A-1 individual with the NOR active distally on one telocentric chromosome 6. Bottom two rows: Chromosomes 2 through 6 from silver stained cells of triploid clone C-1 (C) and a tetraploid (4X). In all cases, the NOR's were observed only on chromosome 6 and were generally active on only one or two chromosomes.


6

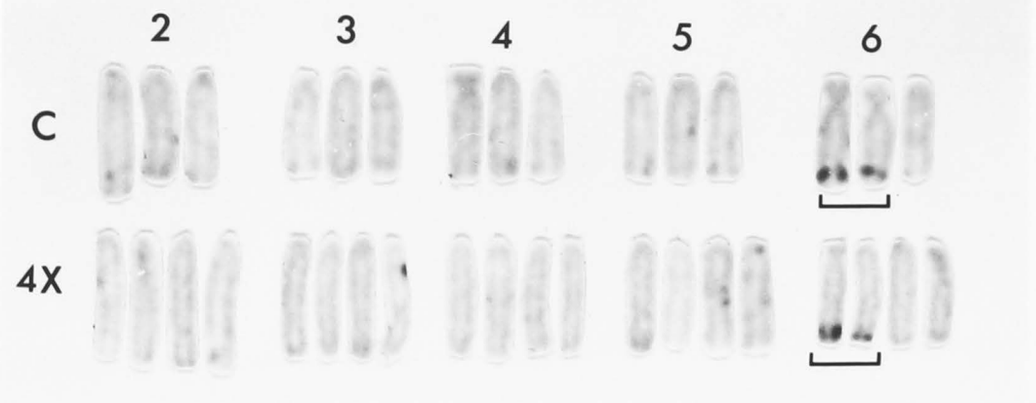

2
3
4
5
6
C
4X

Figure 3.16 (A-D) The results of *in situ* hybridization of an 18 + 28S ribosomal DNA probe from *Drosophila*. In each case the arrows indicate the sites of hybridization. A) a B-1 triploid *H. binoei* from Leonora (loc. no. 40, Table 3-5). B) an A-1 triploid *H. binoei* from Ross River (loc. no. 1, Table 3.5). C-D) two cells from the Randalls Siding tetraploid (loc. no. 28, table 3.5). Note that the triploid individuals have three sites of 18 + 28S ribosomal genes whereas, in the tetraploid, there is one site of strong hybridization (on the submetacentric chromosome 6) and another very weakly hybridizing site on one of the three telocentric 6 chromosomes.

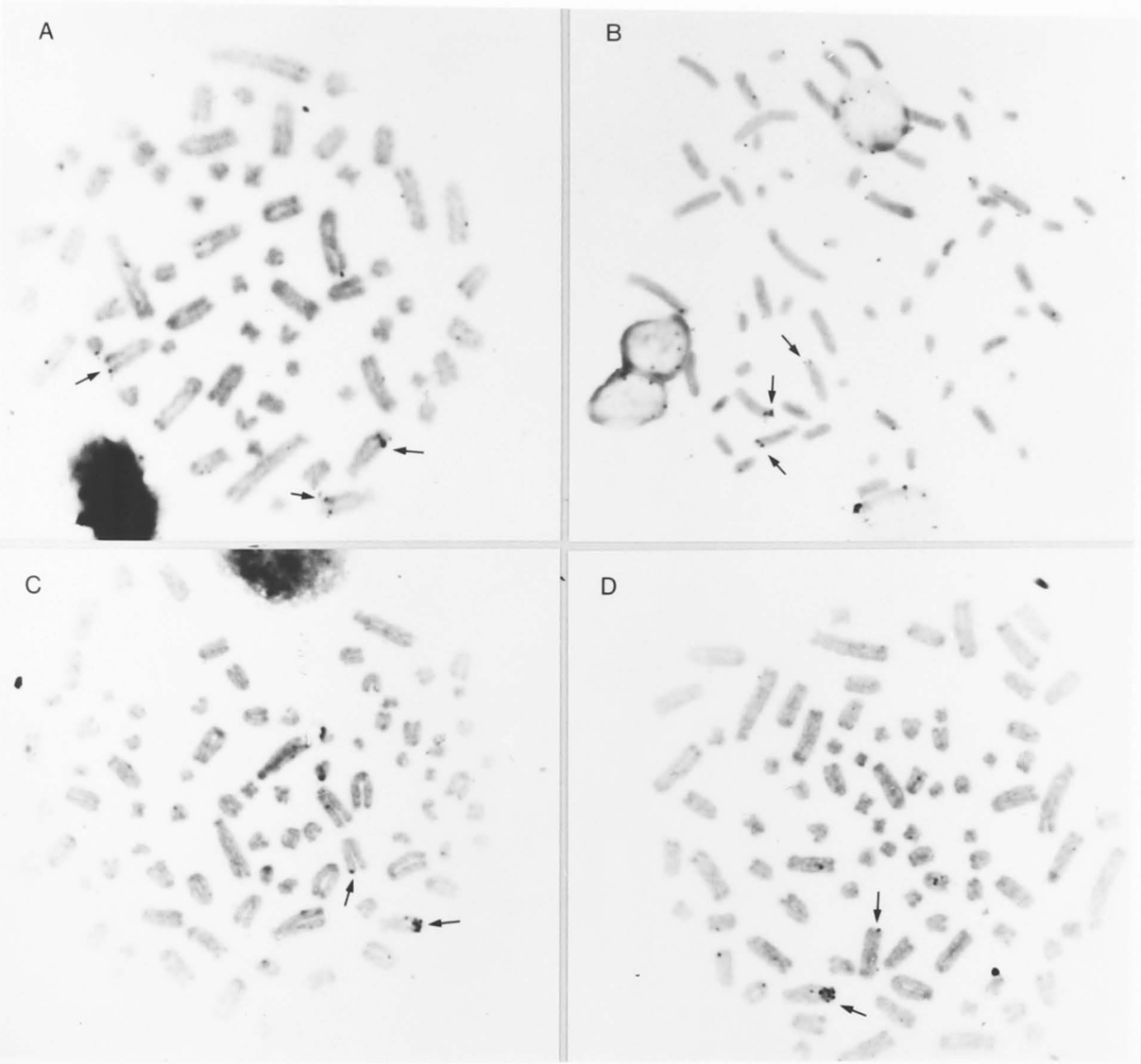

A
B
C
D

Figure 3.17 (A-F) Standard karyotypes of the geographically restricted triploid clones of H. binoei. In each case arrow-heads indicate the position of the secondary constrictions and arrows show the acrocentric member of chromosome 2. A) Clone A-2 with a single acrocentric chromosome 2. B) Clone A-3 which also has a single acrocentric chromosome 2 but also has secondary constrictions on both telocentric members of the chromosome 6 triad. C) Clone B-2 with a single acrocentric chromosome 2, two submetacentric 6's and one telocentric 6 which consistently bears a distal secondary constriction. D) Clone B-3 which has the same heteromorphisms as clone B-2 but never shows a distal secondary constriction on the telocentric 6. E) Clone B-1b. This is actually a derived clone in which the secondary constriction of one submetacentric 6 has been greatly enlarged. F) The karyotype of the single individual representing clone D. Note that all members of the chromosome 6 triad are submetacentric. Only one representative of chromosome 2 consistently had short arms although in this preparations, minute arms are also visible on some other members of the complement.


A
2
6
2
2
6
6
B
6
2
2
6
6
2
C
6
2
6
2
2
6
D
6
2
6
6
2
2
E
6
6
6
F
2
2
6
6
2
6

(A-2,3; B-2,3) had restricted geographic distributions (Fig. 3.12) and were only identified from between one and three localities (Table 3.5).

Clones A-2 and A-3 were identical in respect of having one acrocentric and two telocentric representatives of chromosome 2, and one submetacentric and two telocentric chromosome 6's. However, clone A-3 had secondary constrictions present on both of the telocentric 6's whereas clone A-2 never showed any such constrictions (Fig. 3.17 A, B). Since at least 10 metaphase spreads were examined from each individual, it is extremely unlikely that the reason for this distinction lies in effect of dosage compensation. If this was the case, at least one of the telocentric 6 chromosomes would be expected to have a secondary constriction in clone A-2 and this was not observed.

Clones B-2 and B-3, as their names imply, had two submetacentric and one telocentric chromosome 6's in addition to the chromosome 2 heteromorphism described above. Once again, these clones differed in respect of the presence of a secondary constriction on the telocentric chromosome 6; this was present in clone B-2 and absent in B-3 (Fig. 3.17).

The C-banding analysis of representatives of these clones revealed a compex series of heteromorphisms (Fig. 3.18). In the A-2 and A-3 clones a single unbanded acrocentric chromosome 2 was identified and in clones B-2 and B-3 the same chromosome was present but one of the other two chromosome 2's also lacked an interstitial band. Given the small size of the short arm it was difficult to ascertain from these preparations if the acrocentric morph of chromosome 2 was the result of a pericentric rearrangement or heterochromatic addition (Fig. 3.18).

Figure 3.18 C-banding of chromosomes 1 to 6 of the geographically restricted clones. With the exception of clone D, each of these is highly heteromorphic. The arrows indicate the acrocentric variants of chromosome 2 which invariably lacks an interstitial C-band. Interstitial C-bands were also weak or absent (underlined) in other variants of chromosomes 2, 3 and 5 in these clones.

o = overlap.


A – 2
A – 3
B – 2
B – 3
D
1
2
3
4
5
6

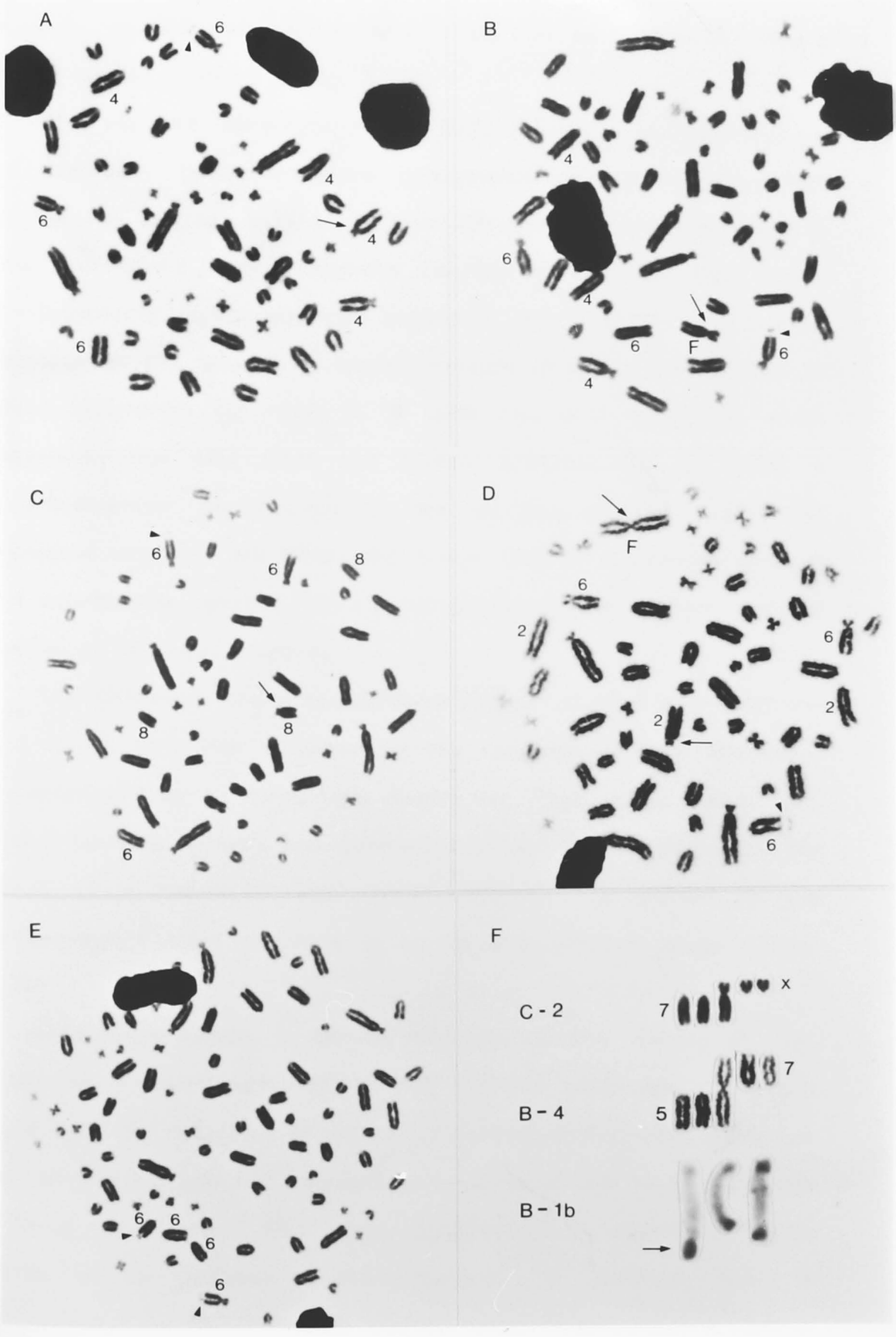
For chromosome 3, the A-2 and A-3 clones had interstitial bands on all three elements however the heteromorphism that was observed in the various A-1 clones was not as evident (cf. 3.18 and 3.14). In contrast the B-2 and B-3 clones had two copies of chromosome 3 with a very weak interstitial band relative to that in the third member (Fig. 3.18). In chromosome 4 of these clones, the number of homologues with a strong procentric band was again variable. The chromosome 5 complement had one or two unbanded members (clones A-2 and B-2,3 respectively) or in the case of clone A-3 displayed the heteromorphism typical of the A-1 group of clones (cf. Figs. 3.18 and 3.14). For chromosome 6, these clones presented the heteromorphisms expected on the basis of standard karyotypes.

One other clone, represented by a single individual from Nanutarra (locality number 53, Figure 3.12), was unique amongst the triploids examined in that it was homozygous for the submetacentric morph of chromosome 6 (Fig. 3.17F). However, this clone (clone D) was heteromorphic for chromosome 2 with one acrocentric and two telocentric members (Fig. 3.17F, 3.18). C-banding analysis of this individual showed that it was unusually homozygous with the only observed heteromorphism being for the acrocentric chromosome 2 (Fig. 3.18).

Derived clones

On the basis of their karyotypes, five clones were considered to be derived from other clones. Of these, two represented changes in heterochromatin (B-1b, Fig 3.17E; C-3, Fig. 3.19A), one was the result of a pericentric rearrangement (B-5, Fig. 3.19C), and two were due to Robertsonian fusions (C-2, Fig. 3.19B; B-4, Fig.

Figure 3.19 (A-D) Standard karyotypes of the chromosomally derived triploid clones of H. binoei. Arrow-heads indicate the position of secondary constrictions. A) Clone C-2 which has two acrocentric representatives of chromosome 4 and two submetacentric morphs of chromosome 6. The arrowed chromosome 4 is the modified chromosome (see text). B) Clone C-2 which has the chromosome 4 and 6 markers of clone C-1 but in addition, carries a large submetacentric chromosome (arrowed) which is the result of Robertsonian fusion (see also F). C) Clone B-5 in which one member of the chromosome 8 triad is acrocentric while the other two have the standard telocentric morphology. D) Clone B-4 which has the chromosome 2 and 6 markers of clone B-2 and also carries a large metacentric chromosome derived by Robertsonian fusion (arrowed, and see F). E) The standard karyotype of the tetraploid from Rawlinna. As is the case for the other tetraploids (Fig. 3.11D), chromosome 6 is present as one submetacentric and three telocentric members. However, note that in this tetraploid, one of the telocentric 6's has a distal secondary constriction. F) Cut out chromosomes showing the homologies of the novel centric fusions in clones C-2 (B) and B-4 (D). At the bottom of F, the C-banded chromosome 6 triad from the derived clone, B-1b, is shown. Note the enlarged block of C-band heterochromatin (arrowed) which corresponds to the enlarged secondary constriction of the submetacentric 6 (see Fig. 3.17E).


A
6
4
4
6
4
6
B
4
6
4
6
F
6
4
C
6
6
8
8
8
6
D
F
6
2
6
2
2
6
E
6
6
6
6
F
C - 2
7
x
B - 4
5
7
B - 1b

3.19D). In each case, these clones have only been identified from a single locality (Table 3.5, Fig. 3.12).

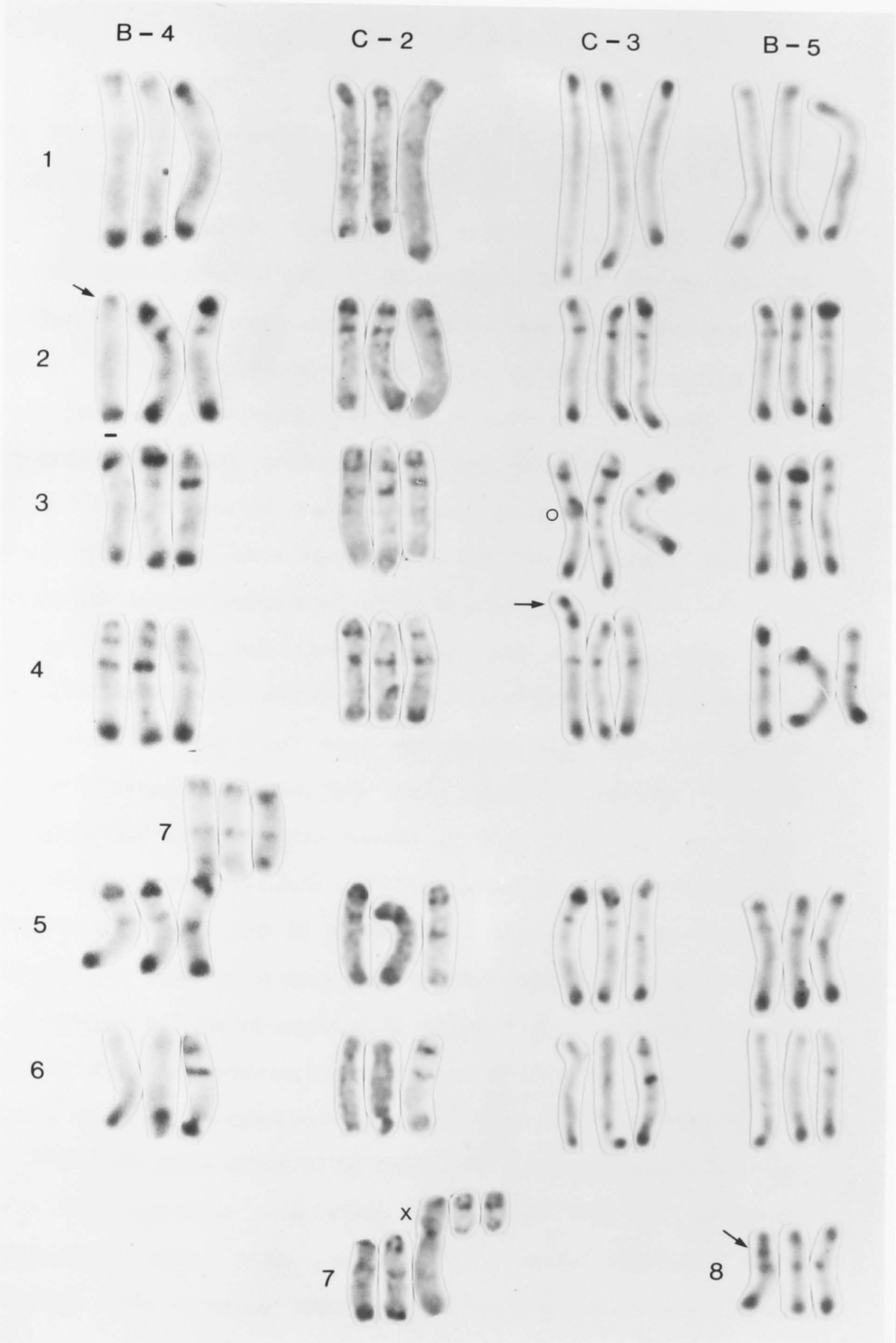
The standard karyotype of the B-1b clone was characterised by an unusually large secondary constriction on the submetacentric chromosome 6 (Fig. 3.17E); a condition that was only observed in four individuals from Doolgunna (locality number 45, Fig. 3.12). Corresponding to the enlarged secondary constriction was a marked increase in the amount of heterochromatin in the distal satellite of these individuals (cf. Fig. 3.19F with Figs 3.14 and 3.18) which suggested that this clone had become differentiated by either a heterochromatin amplification or an increase in the number of ribosomal cistrons (see Miller and Brown 1969 for a demonstration of the relationship between the size of secondary constrictions and the number of ribosomal cistrons).

The C-3 clone had a standard karyotype identical to that of the C-1 clones with the exception of the replacement of a telocentric chromosome 4 by an acrocentric element (cf. Figs. 3.19A and 3.11C). The C-banding analysis has demonstrated that this condition was the result of a heterochromatic amplification on one member of the chromosome 4 triad resulting in an extra acrocentric element (Fig. 3.20).

At a single locality in central Australia (locality number 12, Fig. 3.12) the standard karyotype of the triploids conformed to the B-1 clone with the exception of pair 8. For these chromosomes there was one acrocentric and two telocentrics in place of the normal condition of three telocentrics. This clone, designated B-5, appears to be the result of a pericentric rearrangement; a proposal that is substantiated by the C-banding analysis which demonstrated a shift in

Figure 3.20 C-banding of chromosomes 1 to 6 of the chromosomally derived clones. Where relevant, other members of the karyotype that were involved in rearrangements are also shown. The B-4 and C-2 clones are the result of centric fusions between chromosomes 5 and 7 in the former clone, and chromosome 7 and an unidentified small aerocentric (x) in the latter. The C-3 clone is derived from a C-1 individual through a heterochromatic addition to one member of the chromosome 4 triad (arrowed). Clone B-5 carries a pericentric inversion of one member of the chromosome 8 triad, which has resulted in a shift in the position of the centric C-band (arrowed).

o = overlap.


B – 4
C – 2
C – 3
B – 5
1
2
3
4
7
5
6
x
7
8

the position of the centric heterochromatin in the derived chromosome (Fig. 3.20).

The two remaining clones, B-4 and C-2, departed from the typical triploid karyotype of 2n = 3X = 63 in having only 62 chromosomes. In each case, an extra biarmed chromosome was present (Figs. 3.19B,D,F) and the C-banding analysis has demonstrated the identity of the chromosomes involved in the Robertsonian fusions responsible for the karyotypic changes (Fig. 3.20). In the case of the C-2 clone, chromosome 7 and a small acrocentric element were involved and for the B-4 clone, the large metacentric chromosome is the product of a 7 + 5 fusion.

In each case the derived clones are sympatric with other karyotypically distinct clones (Table 3.4) and in several instances the modified clones may have been derived in situ. On the basis of standard karyotypes, the B-5 clone could be derived from the sympatric B-1 clone (locality number 12, Fig. 3.12) and the C-2 and C-3 clones are also sympatric with their presumed progenitor, the C-1 clone (localities 42 and 23 respectively, Fig. 3.12). However, the C-2 and C-3 clones must have been derived from a C-1 clone in which one member of the chromosome 4 triplet had a strong procentric C-band and this condition has not been observed in the C-banded clone C-1 individuals examined to date (cf. Figs. 3.20 and 3.14).

The B-1b clone observed at Doolgunna (locality number 45, Fig. 3.12) was sympatric with clone A-1b rather than its presumed descendant; clone B-1a. However this latter clone has been identified from sampling sites in close geographic proximity (Fig. 3.12) and it is quite possible that further sampling at Doolgunna would reveal the progenitor clone.

If standard karyotypes alone were considered the B-4 clone would appear to be derived from the sympatric B-2 by the 5 + 7 centric fusion but the C-banding analysis reveals that this is not the case. As Figure 3.20 shows, the derived clone has interstitial bands on two of the three chromosome 2's and all of the representatives of chromosome 5, whereas clone B-2 has only one chromosome with an interstitial band in each triplet.

From these considerations, it is clear that further clones could well be discovered by C-banding analysis and that the 17 clones identified so far may only be a subset of the total clonal diversity in H. binoei. Despite this limitation, 20 of the 54 localities sampled proved to be polyclonal even though the number of individuals C-banded at each site was very small (Table 3.5, Fig. 3.12).

(iii) The origin of parthenogenesis in Heteronotia

The existance of parthenogenetic reproduction in Heteronotia is demonstrated by two facts. Firstly, triploidy was observed in 189 females but no males which, in itself, constitutes strong evidence for subsexual reproduction (Maslin, 1971). Secondly, extensive collecting in the region to the immediate south of Alice Springs (N = 44) and in a large area to the north and north-west of Kalgoorlie (N = 33) has not revealed any males and the absence of males from this area has been further substantiated from examination of museum specimens (Fig. 3.22, Lindenmayer, Nix and Moritz, unpublished). Since the sex ratios of H. binoei in exclusively bisexual populations are generally even (Bustard 1968b; Moritz pers. obs.), the absence of males from these areas clearly excludes sperm-dependant modes of subsexual reproduction such as gynogenesis and hybridogenesis (see

section 3.1). Given this demonstration of parthenogenesis, the next issue of concern is to define the mode of origin of this mechanism of reproduction.

The cytogenetic evidence presented above clearly indicate a hybrid origin of parthenogenesis in Heteronotia. The standard karyotypic data alone provide conclusive evidence for this hypothesis, since all of the triploid females were heterozygous for one or more karyotypic rearrangements. When the combined karyotypic data for the diploid and triploid H. binoei are considered, it is evident that the extensive clonal diversity of the parthenogenetic biotype of Heteronotia is primarily due to multiple hybrid origins.

This process can be accurately reconstructed for the widespread clones (A-1, B-1 and C-1). The most parsimonious explanation for the presence of the heteromorphism of chromosome 6 in all of the clones and the additional chromosome 4 heteromorphism in clone C-1, is that hybridization between the A6 and SM6-1 cytotypes resulted in an allodiploid which was heterozygous for the chromosome 6 marker and was capable of parthenogenetic reproduction. Backcrossing between this allodiploid (producing unreduced eggs) and the A6 and SM6 cytotypes would account for the observed heteromorphisms. Specifically, clone A-1 would be the result of backcrossing between the allodiploid and an A6 male whereas clones B-1 and C-1 would have been generated by crosses to the SM6-1 and SM6-2 cytotypes respectively.

On the basis of standard karyotypes, the A6 cytotype involved in the hybridization events leading to these widespread clones is invariably the central and western form which lacks the distal secondary constriction on chromosome 6. Note also that the SM6

cytotype implicated in these events could be exclusively the SM6-2 form since these populations were defined by the presence of a polymorphism for an acrocentric:telocentric combination of chromosome 4. The postulated allodiploid parthenogen has not been identified despite intensive collecting in central and western Australia and if it still exists, it must have a very restricted geographic distribution.

The hypothesis of hybrid origin is substantiated and extended by the C-banding analysis of these clones. The observed heteromorphisms of chromosomes 3, 5 and 6 amongst these clones can only be accounted for hybridisation between the CA6 karyomorph and the SM6 cytotype. (cf. Figs. 3.6, 3.8 and 3.14). Furthermore, the fact that all of the chromosome 2's in these clones bear interstitial bands, excludes the SM6-1a and SM6-1b karyomorphs. Also, both of the SM6-3 karyomorphs can be eliminated on the basis of their C-banding patterns and morphology of chromosomes 4, 5 and 6 (cf. Figs. 3.7, 3.8 and 3.14). If follows that the populations involved in the hybridisation events must have been the CA6 and SM6-2 karyomorphs.

Examination of the chromosome 4 C-band heteromorphism provides further information on the origin of these clones. Amongst west Australian populations of both the CA6 and SM6-2 karyomorphs there existed polymorphisms for the presence of a chromosome 4 with a strong procentric band (Figs. 3.2, 3.4). However, in the central Australian populations, this variant of chromosome 4 was absent in the CA6 populations sampled (Fig. 3.2). Thus, all of these clones could have arisen in western Australia while clones A-1b and A-1c, in which one or both of the CA6 genomes must have had a chromosome 4 with a strong procentric band, could not have originated in central

Australia. Since both of these clones are present in central Australia (Table 3.4, Fig. 3.12) it follows that they have colonised this area after their origin in western Australia.

This hypothesis rests on the presumption that the different combinations of chromosome 4 variants in these clones is not attributable to recombination. If rare recombination events are responsible for these patterns of clonal diversity, then the other members of the complement should also be affected and this does not appear to be the case. For example occasional recombination should result in rare genotypes of the following forms; homozygosity for chromosome 6, submetacentric 6's with interstitial bands, the presence of two submetacentric 6's and two acrocentric 4's (recombination on chromosome 4 of clone C-1) and clones with heteromorphisms of chromosomes 3 and 5 that are inconsistent with the chromosome 6 constitution. With the exception of a single individual homozygous for the submetacentric 6 (clone D), these genotypes have never been observed.

A model for the origin of these widespread clones which is based on multiple hybridisation events and which accounts for all of the observed structural and C-band heteromorphisms is presented in Figure 3.21. In addition to the A-1, B-1 and C-1 clones identified to date, the model includes a variant of clone C-1 which has a single chromosome 4 bearing a strong proximal band which was identified in the derived clones; C-2 and C-3. Rare tetraploids, which are the result of insemination of triploid females by males, are also depicted and will be discussed below.

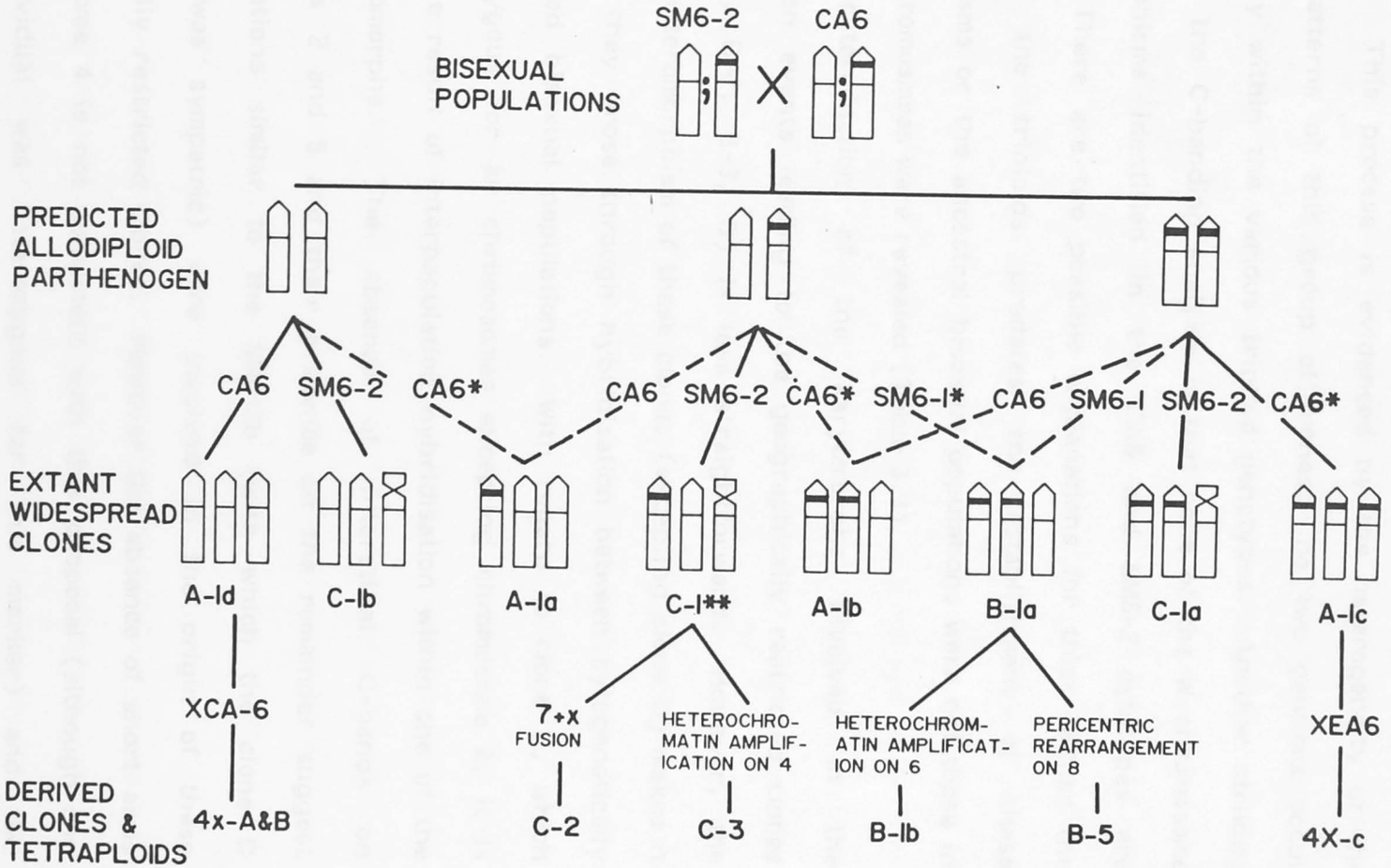
An important feature of the model is that multiple hybridisation events between the ancestral bisexual populations are required in

Figure 3.21

Tentative model for the origin of the widespread triploid clones of H. binoei (A-1, B-1 and C-1), based on an analysis of variation chromosomes 4 and 6. Only the chromosome 4 variants are shown. To account for the observed distribution of variants of chromosome 4, the ancestral bisexual populations (CA6 and SM6-2) must have been polymorphic. Multiple hybridisation events at this stage must have produced a minimum of three allodiploids capable of parthenogenetic reproduction. The observed triploid clones are postulated to have arisen through backcrossing of these allodiploids to genetically distinct bisexual individuals. The dotted lines indicate alternative backcrosses which could have produced the clones. The derivation of the three tetraploids (4X - A,B,C - see Fig. 3.23) by further backcrossing is shown at the bottom of the figure. Additionally, the chromosome rearrangements leading to four of the five derived clones are depicted.

* these gametes carried a morph of chromosome 4 with a strong procentric band

** this clone has not been identified but is presumed to have existed on the basis of the chromosome 4 constitution of the derived clones, C-2 and C-3.


SM6-2
CA6
BISEXUAL POPULATIONS
PREDICTED ALLODIPLOID PARTHENOGEN
CA6
SM6-2
CA6*
CA6
SM6-2
CA6*
SM6-1*
CA6
SM6-1
SM6-2
CA6*
EXTANT WIDESPREAD CLONES
A-Id
C-Ib
A-Ia
C-I**
A-Ib
B-Ia
C-Ia
A-Ic
XCA-6
7+x FUSION
HETEROCHROMATIN AMPLIFICATION ON 4
HETEROCHROMATIN AMPLIFICATION ON 6
PERICENTRIC REARRANGEMENT ON 8
XEA6
DERIVED CLONES & TETRAPLOIDS
4x-A&B
C-2
C-3
B-Ib
B-5
4X-c

addition to the repetitive backcrossing to the various bisexual cytotypes. This process is evidenced by the heterogeneity of the C-band patterns of this group of clones; no two genomes occur consistently within the various triploid genotypes. Another striking feature of the C-banding analysis is that none of the W chromosome heteromorphisms identified in the CA6 and SM6-2 cytotypes are present. There are two possible explanations for this; either the origin of the triploids predates the establishment of these polymorphisms or the ancestral bisexual populations were not those in which W chromosomes were revealed (Table 3.3).

The determination of the karyomorphs involved in the hybridisation events leading to the geographically restricted clones (A-2, A-3, B-2, B-3, D) is less straightforward. However, the extensive heteromorphism of these clones (excepting clone D) makes it clear that they arose through hybridisation between cytogenetically differentiated bisexual populations. With regard to clone D, which was homozygous for all chromosomes excepting chromosome 2, it is possibly the result of interpopulation hybridisation within one of the SM6 karyomorphs. The absence of interstitial C-bands on chromosomes 2 and 5 and their presence on the remainder suggest that populations similar to the SM6-3b (with which the clone D individual was sympatric) were involved in the origin of these geographically restricted clones. However the absence of short arms on chromosome 4 is not consistent with this proposal (although one SM6-3 individual was heterozygous for this marker) and the acrocentric variant of chromosome 2 has not been identified in any of the bisexual populations studied to date.

That this variant of chromosome 2 has not been found in the SM6 populations so far studied, obviously precludes a precise description of the hybridisation events that resulted in these restricted clones. In clones A-2, B-2 and B-3 the acrocentric chromosome 2 and another member of this triad lacking an interstitial band (clones B-2 and B-3 only), are associated with morphs of chromosome 5 that also do not have interstitial C-bands (Fig. 3.18). This again implicates a SM6 karyomorph similar to the SM6-3 in their origin. However, excluding the acrocentric chromosome 2, the SM6 complement of the A-3 clone is most similar to the SM6-1b karyomorph (cf. Figs. 3.18 and 3.7). The C-banding data indicate that further analysis of the western SM6 populations is required before the hybridisation events that produced the restricted clones can be defined. However, they do demonstrate one important point; the SM6-2 karyomorph which was involved in the widespread clones (A-1, B-1, C-1) did not play a part here.

The A6 cytotype genomes found in A-2 and B-3 clones correspond precisely to the CA6 karyomorph (cf. Figs. 3.18 and 3.5) but this was not the case for the A-3 and B-2 clones. In these, a distal secondary constriction was present on chromosome 6, while the C-banding pattern was the same as for the CA6 karyomorph; a combination of markers that have not been so far identified in bisexual populations.

When the C-band patterns and structural heteromorphisms of these five geographically restricted clones are compared (Fig. 3.18) it is clear that, once again, no two genomes occur consistently between clones. This observation further substantiates the role of multiple hybridisation and repetitive backcrossing in generating the extensive clonal diversity found within the parthenogenetic biotype of Heteronotia.

(iv) Cytogenetic diversification within the parthenogenetic lineages

The approach adopted in this study was to undertake a detailed comparative analysis of chromosome variation in the triploid parthenogens and their progenitors; the diploid bisexual populations. As a corollary to this, it is assumed that the heteromorphisms of the triploids that are shared by their probable diploid ancestors represent a conservation of the variation induced by multiple hybridisation and repeated backcrossing.

On this basis, five cytogenetically derived clones were identified and these were the result of heterochromatin amplification, possible ribosomal cistron duplication, pericentric rearrangment and Robertsonian fusions. These last of these modifications, which were present in clones C-2 and B-4, are of particular interest since this mechanism of chromosomal change is absent from the diploid bisexual Heteronotia. The significance of this observation is discussed in more detail in the concluding section of this chapter.

In the case of the geographically restricted clones it is difficult to determine if any of the heteromorphisms that have not been identified from bisexual Heteronotia are due to chromosomal mutation following the origin of parthenogenesis. For example, it is possible that the B-2 and B-3 clones, which have virtually identical C-banding patterns have become differentiated by a change in the behaviour of the telocentric chromosome 6 NOR. However, if this is the case, the same mutation must have occurred independantly in the A-3 clone which has a distinct array of structural and C-band heteromorphisms to those of the B-2 and B-3 clones. The same argument can be applied to the acrocentric morph of chromosome 2. It is therefore suggested that the heteromorphisms of these clones are attributable to

hybridisation events between A6 and SM6 karyomorphs; some of which have not been identified. Clearly, further cytogenetic analysis of bisexual H. binoei from western Australia is needed to substantiate this claim.

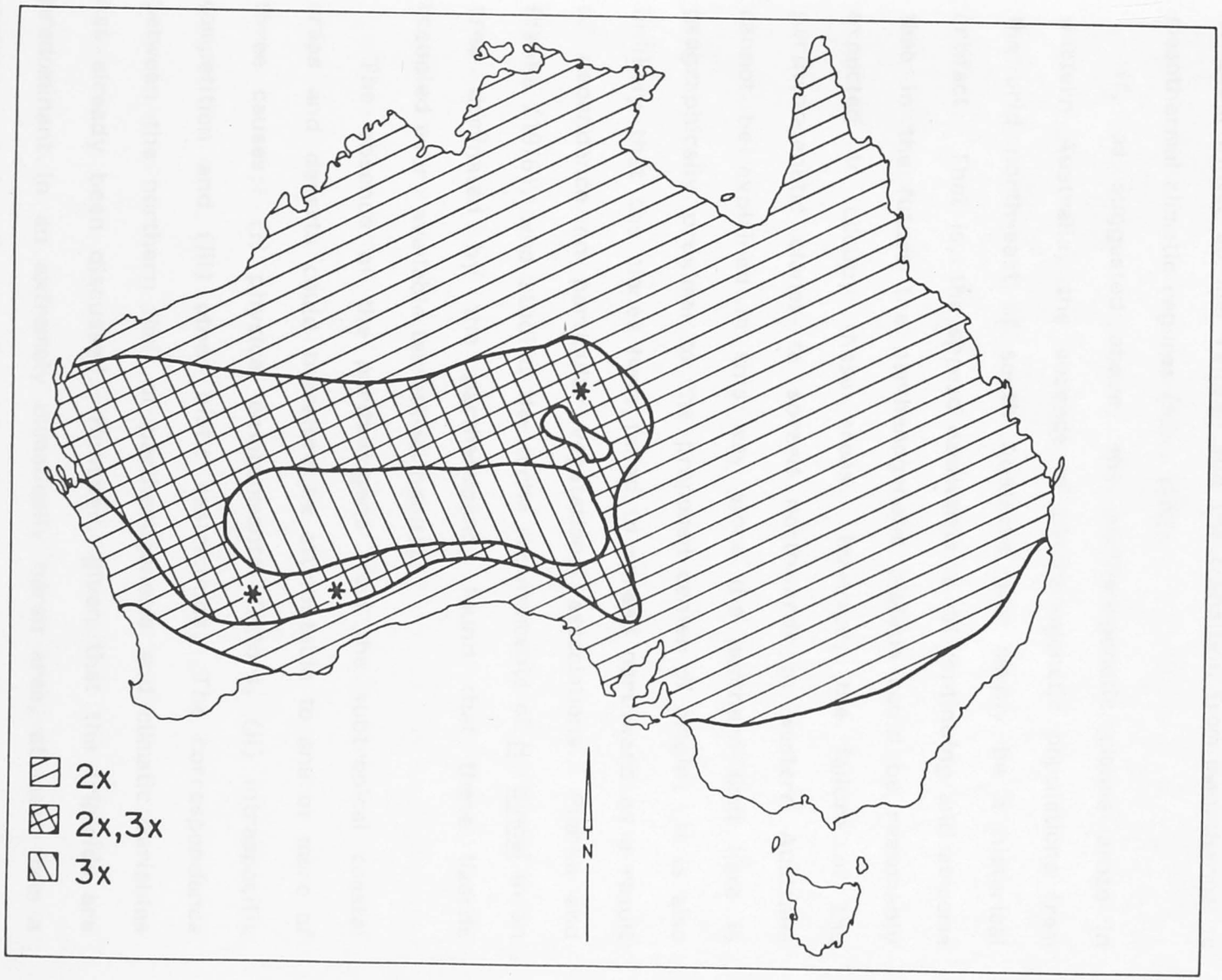
(v) Geographical parthenogenesis in Heteronotia

The comparative zoogeography of bisexual and parthenogenetic H. binoei is to be the subject of a detailed study (Lindenmayer, Nix and Moritz, unpublished) and at this stage only very crude climatic and biotic correlates can be offered. The total distribution patterns shown in Figure 3.22 are based primarily on the cytogenetic evidence presented above and are confirmed by analysis of sex ratios in museum specimens. However, the latter data must be treated with caution since, while the presence of males proves that bisexual populations are present, it does not of course, demonstrate the absence of parthenogenetic females. This restriction applies to the Great Sandy Desert in particular since there are no karyotypic data for H. binoei from this region. In this case the presumed absence of parthenogenesis is based only on the presence of males in museum collections.

Two features of this distribution pattern are of particular interest. Firstly, the parthenogenetic biotype as a whole is restricted to arid regions and moreover, the northern limit of its distribution approximates the tropic of Capricorn (Fig. 3.22). This northern limit is well defined in the coastal area of western Australia, and up to 400 km inland, as well as for central Australia (including the Tanami desert) but the status of the populations of the Great Sandy Desert is not as clear. The proposed northern limit

Figure 3.22

Map of Australia showing the tentative distribution patterns of diploid and triploid H. binoei. No males of this species have been found in a small area of the southern Northern Territory and males also appear to be absent from a broad band extending from north-west South Australia to the Kalgoorlie area in western Australia. Note that, despite the extremely broad area of overlap between the distributions of the diploid and triploid taxa, the production of tetraploids (*) is a rare event.


2x
2x,3x
3x
N

coincides closely with important physical parameters such as the line of equal winter-summer rainfall and the transition from megathermal to mesothermal climatic regimes (Nix, 1982).

If, as suggested above, the parthenogenetic clones arose in western Australia, the absence of parthenogenetic populations from the arid north-east of south Australia may simply be a historical artifact. That is, the spread eastwards is till continuing and at some time in the future the parthenogenetic biotype could be reasonably expected to occupy these areas. However, the failure of the parthenogenetic clones to spread northwards in western Australia cannot be explained in this way since the northern limit here is geographically proximal to the proposed center of origin. It is also unlikely that the clones have failed to spread northward as a result of dependance on particular vegetational associations. Pianka and Pianka (1976), who studied the niche requirements of H. binoei in an area dominated by the parthenogens, found that these lizards occupied every available terrestrial habitat.

The absence of the parthenogens from the subtropical coastal areas and deserts could therefore be attributable to one or more of three causes; (i) physical environmental factors, (ii) intraspecific competition and (iii) other biotic restrictions. The correspondance between the northern limit of parthenogenesis and climatic variables has already been discussed. However, given that the triploids are predominant in an extremely climatically harsh area, at least from a anthropocentric point of view (Fig. 3.22), it is difficult to conceive of physical environmental factors per se restricting the spread of parthenogenesis. This issue could be resolved through comparative studies of the environmental physiology of the subsexual and bisexual

populations near this northern boundary with particular emphasis on water relations (e.g. the studies by Bradshaw 1981).

Intraspecific interactions may also be important in restricting the distribution of the triploid H. binoei. The life history patterns of lizards generally vary with climatic conditions (e.g. Tinkle et al. 1970; Tinkle and Ballinger 1972; Bradshaw 1981) and it is possible that the subtropical bisexual populations of H. binoei may have a higher reproductive rate than their southern arid zone counterparts. If this were the case, then the parthenogenetic populations may gradually lose their inherent demographic advantage as they extend northwards. This hypothesis is readily amenable to empirical study and analyses of the comparative reproductive biology of the sexual and parthenogenetic forms of H. binoei and of temperate and subtropical bisexual populations are urgently required.

The third possible restriction on the distribution of the triploids concerns other forms of biotic interactions. The most important of these would be; (i) interspecific competition and (ii) host-pathogen relationships. The former component is difficult to evaluate but it is notable that the area occupied by the parthenogenetic biotype corresponds to the region of maximal species density of gekkonid lizards in Australia (Cogger and Heatwole 1981). However to infer the level of interspecific competition from this observation in the absence of species density data is not warranted. It may be relevant that in natural habitats in the western half of the distribution of the triploids, H. binoei is quite rare relative to other species of geckos (Pianka and Pianka 1976; Pianka pers. comm.). No relevant data can be presented from the current study since specimens were generally obtained from under sheets of iron in rubbish dumps where Heteronotia is extraordinarily abundant.

The second factor, host-pathogen relationships, is a subject for which no data exist and once again, comparative studies of the pathogen load of subsexual and sexual populations and between bisexual populations from the subtropical and southern deserts are necessary.

The second major feature of interest in the distribution patterns shown in Figure 3.22, is the widespread sympatry between the diploid bisexual populations and the triploid clones of H. binoei (see also Tables 3.3 and 3.5). Indeed, the area in which H. binoei reproduce exclusively by parthenogenesis is quite restricted and even then may have been overestimated since the sampling conduced in, and the availability of museum specimens from, north-west South Australia and the Great Victoria desert is minimal.

This observation suggests two further areas of future research. Firstly, it is important to investigate the nature of competitive interactions between the bisexual and subsexual populations where they occur in sympatry since this factor is critical to several of the hypotheses for the maintenance of sex discussed in Section 3.1. Secondly, the extent of premating isolation between the parthenogenetic females and the sympatric males of H. binoei requires clarification. Of the 156 H. binoei sampled from localities where the diploids and triploids were sympatric (see Tables 3.3 and 3.5) only three were tetraploids (see below). Since these tetraploids were somatically normal, it is most probable that premating isolation, albeit imperfect, exists between the diploid and triploid populations.

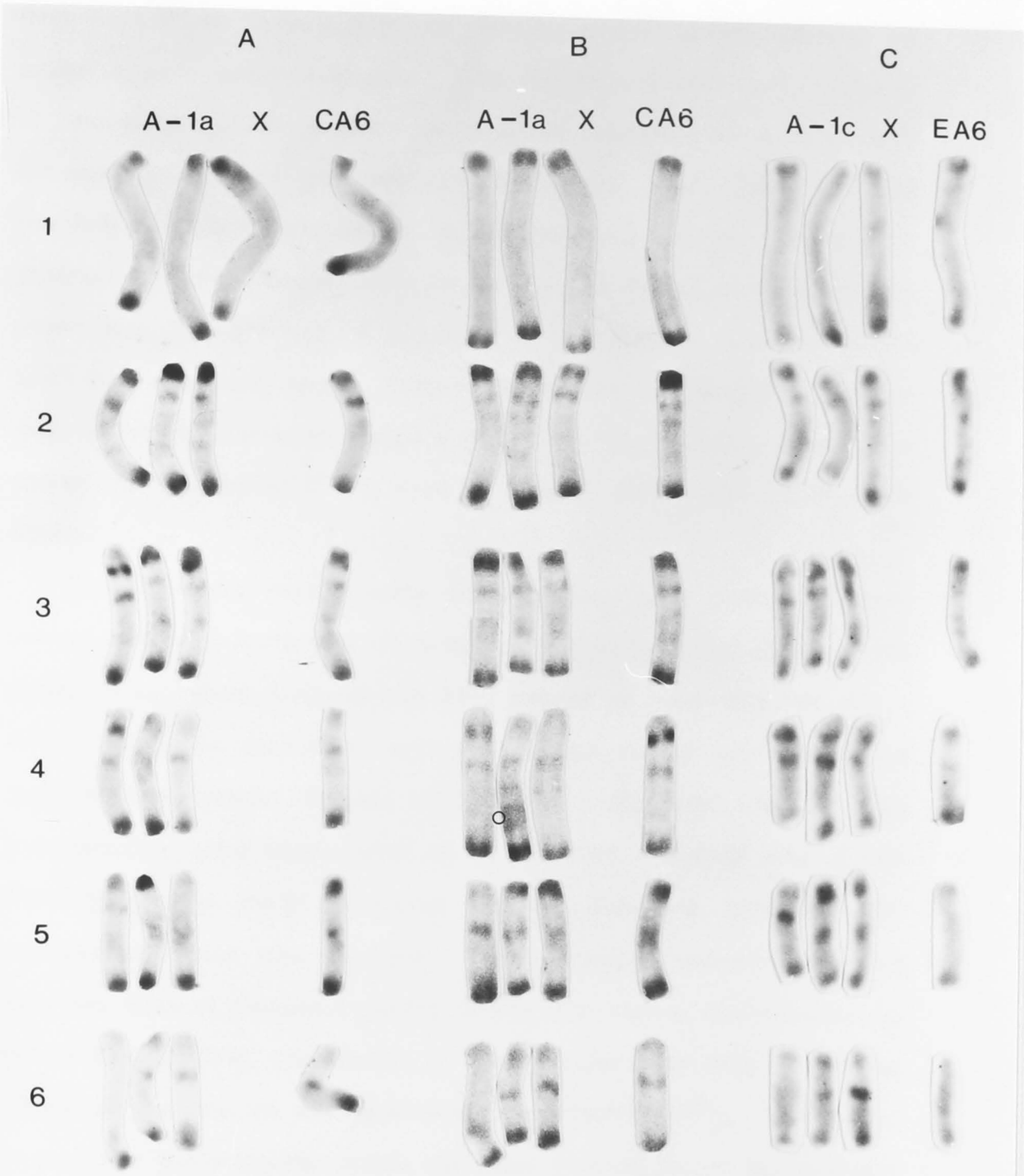
(iv) Tetraploid H. binoei

Three tetraploid females have been identified amongst the H. binoei examined; one from central Australia and two from western Australia (Fig. 3.22). In each case chromosome 6 was present in the form of three telocentric and one submetacentric members. In the tetraploid from Rawlinna (locality number 38, Fig. 3.12) one of these telocentrics had a secondary constriction (Fig. 3.19E) whereas the other tetraploids only expressed secondary constrictions on the submetacentric chromosome 6 (Fig. 3.11D).

A C-banding analysis of these individuals (Fig. 3.23) provides strong support for the proposition that they arose from the insemination of triploid females by sympatric males. At Ross River (locality number 1, Fig. 3.12) clones A-1a and A-1d are sympatric with CA6 diploids and the tetraploid has the C-banding pattern expected for a hybrid between the A-1a clone and a CA6 bisexual (Fig. 3.23A). At Randall's Siding (locality number 28, Fig. 3.12) a sample of only two H. binoei included a triploid of clone A-1a and the tetraploid. The C-banding analysis of this tetraploid (Fig. 3.23B) indicates that it is a hybrid between the A-1a clone and a CA6 male with a proximal C-band on chromosome 4 which is characteristic of H. binoei from areas close to this locality (see Fig. 3.2). The third tetraploid, collected from Rawlinna, was distinguished from the other two by having a secondary constriction on one of the telocentric members of chromosome 6. This suggests that it was the result of insemination of a clone A-1 individual by a male of the EA6 karyomorph. This was verified by C-banding (Fig. 3.23C) which showed that the tetraploid arose from a cross between the A-1c clone and an EA6 male and both of these forms of H. binoei were identified from this locality.

Figure 3.23 (A-C)

C-band patterns of chromosomes 1 to 6 from the three tetraploid females of H. binoei. A+B). Individuals from Ross River (N.T.) and Randall's Siding (W.A.) respectively. C) the tetraploid from Rawlinna (W.A.). The tetraploid karyotypes have been split into the triploid and haploid complements that were presumed to have been involved in the back-crosses. Note the relatively faint C-bands of the EA6 genome which is a characteristic of this karyomorph. = overlap.


A
B
C
A-1a X CA6
A-1a X CA6
A-1c X EA6
1
2
3
4
5
6

Examination of the reproductive tract of the tetraploid female from Ross River showed that the ovaries were atrophied and that the oviducts were underdeveloped. This female was obviously incapable of oogenesis which accords with other reports of hybridisation between established parthenogenetic lineages and their bisexual relatives resulting in sterile polyploids (see section 3.3.1). In contrast to this observation, the tetraploid female from Rawlinna, which was the product of a different hybridisation event (A-1c x EA6) had produced eggs. Examination of the reproductive tract of this individual revealed corpora lutea on the ovaries however it cannot be determined if the eggs that were laid by this female were viable.

The tetraploid females were also examined with respect to their pattern and distribution of ribosomal gene activity. Two females were examined by silver staining and both proved to have only two active NOR's which is consistent with the dosage compensation observed amongst the triploid females (Fig. 3.15). However when in situ hybridisation experiments with an 18 and 28s ribosomal RNA probe from Drosophila were conducted on the Rawlinna tetraploid, an unexpected result was obtained. The tetraploid appeared to have only two sites of ribosomal genes; a site with strong hybridisation on the submetacentric chromosome 6 and another site with far fewer ribosomal cistrons on one telocentric chromosome 6 (Fig. 3.16C, D). In contrast the triploids, which had been exposed to the probe under identical conditions, each maintained the expected three sites of ribosomal genes. It is possible, of course, that more prolonged exposure of the tetraploid cells to the Drosophila probe, or the use of a probe from a more closely related organism, would reveal the existance of ribosomal genes on the other telocentric representatives

of chromosome 6. However, these preliminary studies do suggest that the submetacentric chromosome 6 of the tetraploid has acquired a disproportionately large number of ribosomal cistrons.

The triploid clone from which this tetraploid was derived (A-1a) was not characterised by an unusually large secondary constriction on chromosome 6 (e.g. Fig. 3.11A) but in the absence of *in situ* hybridisation studies of the triploids from this locality (the clone A individual in Figure 3.16B is from central Australia), it cannot be demonstrated that the increase in the number of ribosomal cistrons on the submetacentric chromosome 6 of the tetraploid occurred in that individual.

3.2.2 *Nactus arnouxii* (formerly *Cyrtodactylus pelagicus*)

Nactus arnouxii is a small terrestrial gecko, generally confined to the litter of forest floors and has a widespread distribution from Papua New Guinea and the north-eastern extremity of Australia to the south-central Pacific (Tuamoto Archipelago, Kluge, 1983). On external morphology, this lizard closely resembles *H. binoei* and in fact Russell (1972 cited in Kluge 1983) considered synonymising *N. arnouxii* with *Heteronotia*. Recent studies of the skeletal anatomy of this gecko (Kluge loc. cit.) have led to its removal from the extremely speciose genus *Cyrtodactylus* (which had previously contained 76 species) and its inclusion in a newly erected genus, *Nactus*. Kluge (loc. cit.) also emphasised the distinction between *N. arnouxii* and *Heteronotia* which makes it clear that their general resemblance in external morphology must be due to convergent evolution (see also chapter 7).

Very little is known about the ecology of this species, but from a detailed study of the reproductive biology of several lizard species on American Samoa, Schwaner (1980) made the important observation that all of the 51 individuals examined were female. This finding prompted an analysis of chromosomal and protein variation in this species, the preliminary results of which are reported here.

(i) Bisexual populations - Distribution and cytogenetics

To determine the distribution of the bisexual populations of N. arnouxii, the sex of a large series of preserved specimens in the Australian Museum was determined. This revealed that males of this species occur in Papua New Guinea, Cape York (Australia), the Solomon islands and Vanuatu (see Fig. 3.24). A series of specimens from the bisexual populations of Cape York, Pt. Moresby and Vanuatu were examined cytogenetically and electrophoretically to provide genetic markers for a subsequent study of the parthenogenetic biotype of N. arnouxii.

The cytogenetic analysis demonstrated extensive variation in chromosome number amongst these samples (Table 3.6, Figs 3.25, 3.26) which appears to be primarily, although not exclusively, due to Robertsonian translocations.

It had been previously suggested (Black pers. comm.) that N. arnouxii from Papua New Guinea actually included two species which differed both in their body size and in the number of preanal pores in males. Of the four specimens examined from Pt. Moresby, three were of the "small morph" and one represented the "large morph". Each of the four individuals analysed proved to be heterozygous for a Robertsonian fusion (2n = 39) but the fusion involved differed

Table 3.6

Localities from which Nactus arnouxii were karyotyped together with the results obtained for each sample. The 2n = 35 form (loc. no. 6 and 7) is parthenogenetic. The numbers refer to points on figure 3.24.

TABLE 3.6

LOCALITY	N	CHROMOSOME NUMBER (2n)
1. PT. MORESBY, PAPUA NEW GUINEA	3	39A, het for 1+6 FUSION
2. PT. MORESBY, PAPUA NEW GUINEA	1	39B, het for 6+8 FUSION
3. COOKTOWN, AUSTRALIA	5	38
4. MISSION BEACH, AUSTRALIA	3	38
5. EFATE, VANUATU	3	28
6. SUVA, FIJI	2	35 het for 5 FUSIONS
7. NIUE, COOK ISLANDS	1	35 " " " "

Figure 3.24

Map of the islands of the western Pacific ocean showing the geographic location of the sampled Nactus arnouxii populations. The numbers refer to Table 3.6. This species may also be parthenogenetic on the islands of New Caledonia which are denoted by "P?". Note that the eastern limit of N. arnouxii is well beyond the area sampled (Tuamoto archipelago - see Fig. 3.31).

Symbols: □ 2n = 38

△ 2n = 39A

▽ 2n = 39B BISEXUAL

○ 2n = 28

● 2n = 35 PARTHENOGENETIC

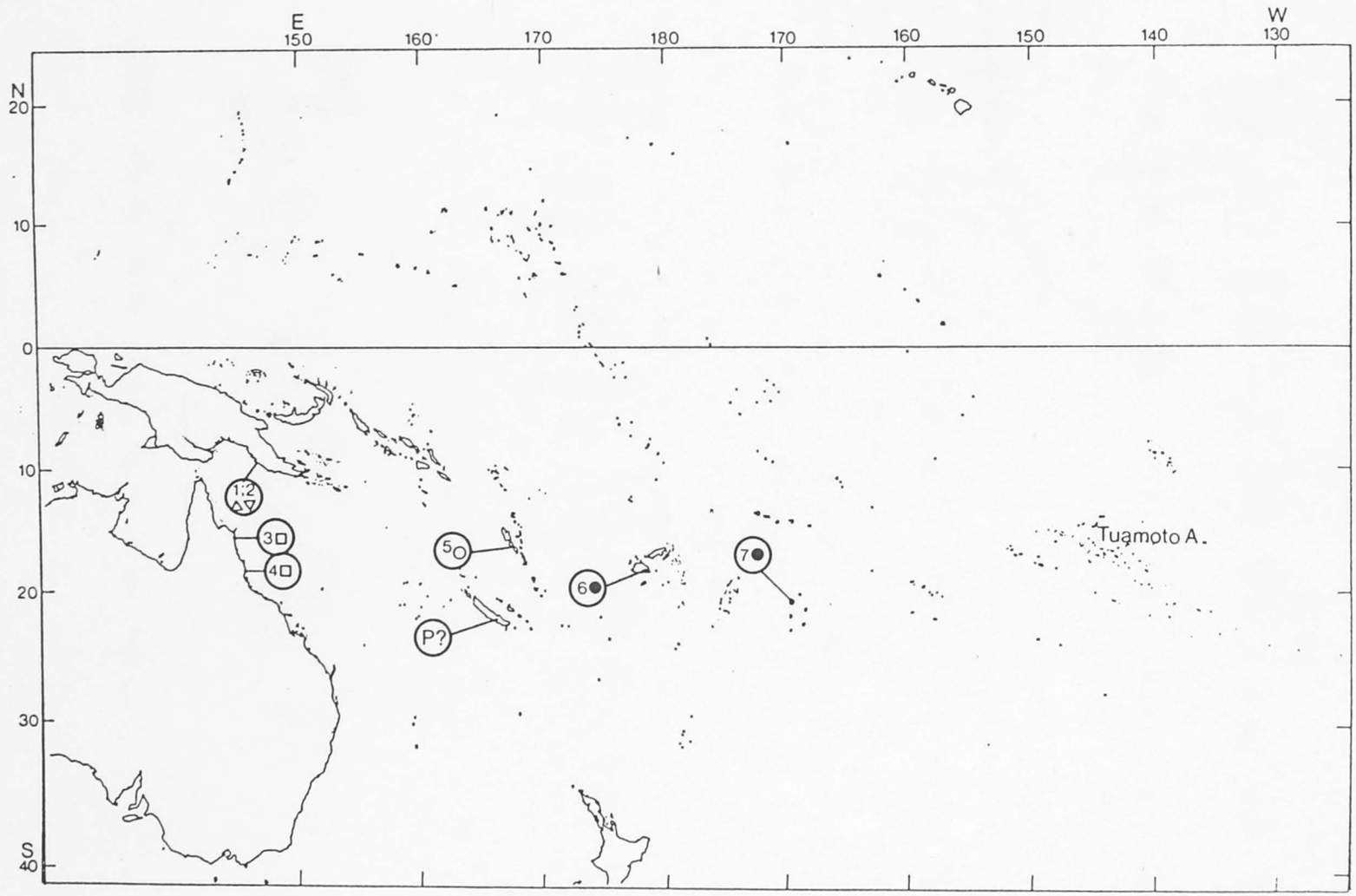

E
150
160
170
180
170
160
150
140
W
130
N
20
10
0
10
20
30
S
40
1;2
3
4
5
6
7
P?
Tuamoto A.

Figure 3.25 (A-D)

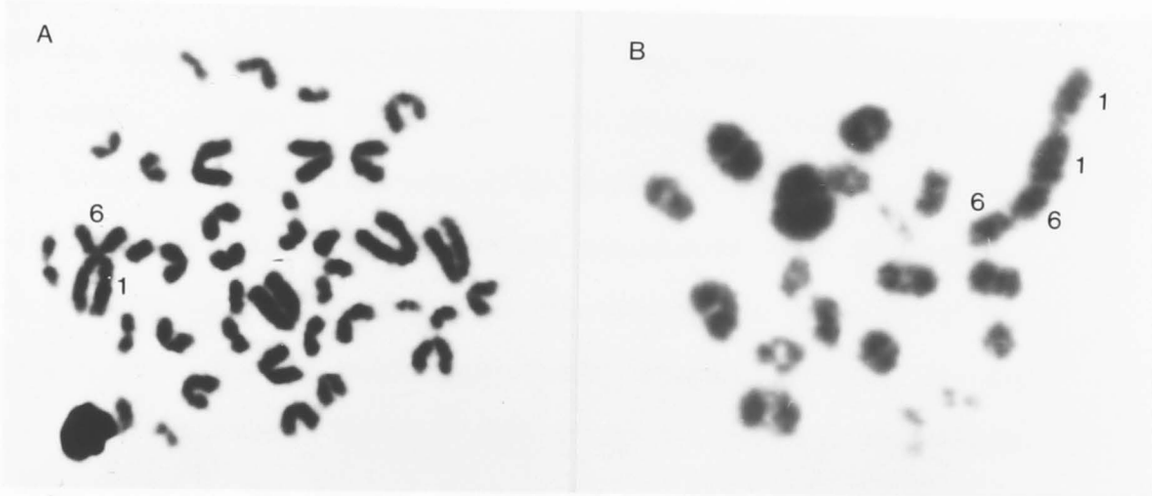
Giemsa stained karyotypes of the chromosomally distinct forms of Nactus arnouxii. A) A 2n = 39B individual from Pt. Moresby, Papua New Guinea. Note the heterozygous Robertsonian fusion. B) The 2n = 38 karyotype typical of N. arnouxii from Cape York, Australia. C) The 2n = 28 karyotype found in the Vanuatu sample of N. arnouxii. D). The highly heterozygous 2n = 35 karyotype of parthenogenetic N. arnouxii from Fiji and Niue.

The chromosome pairs of the 2n = 28 karyotype (C) that are underlined appear morphologically identical to the metacentric, submetacentric and acrocentric chromosomes of the 2n = 35 parthenogen (D).


A
B
C
D

Figure 3.26 (A-B).

Mitotic (A) and meiotic (B) metaphases of the bisexual 2n = 39A form of Nactus arnouxii. Note the heterozygous metacentric A, which is presumed to be a result of centric fusion between the largest and sixth largest telocentrics of the karyotype. This metacentric and its homologues regularly form a trivalent in meiosis (B).


A
6
1
B
1
1
6
6

between the two morphological forms. The large morph individual was heterozygous for a 6 + 8 centric fusion (2n = 39B, Fig. 3.25A) whereas the three specimens of the small morph were all heterozygous for a fusion between chromosomes 1 and 6 (Fig. 5.26A). The assignment of individual chromosomes to these fusion events is based on their size alone and this scheme must therefore be considered tentative until a G-banding analysis is conducted. However, C-banding of the 2n = 39B karyotype (Fig. 3.27A) has demonstrated that the metacentric chromosome of this complement is the result of a centric fusion rather than heterochromatic addition.

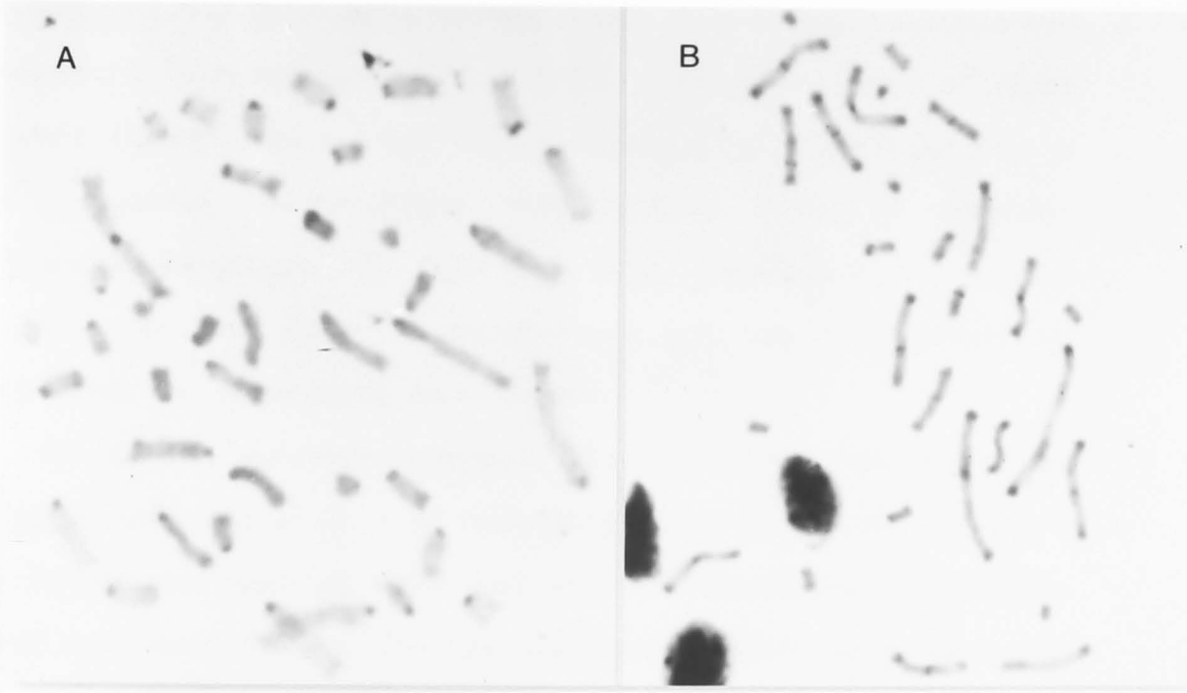
Meiosis was studied in one male which was heterozygous for the 1 + 6 fusion. In every metaphase-1 cell examined (N = 35 cells) a fusion trivalent was observed (Fig. 3.26B). However, in the preparation examined, very few second metaphases were present, so that it was not possible to determine the segregation patterns of the trivalent: the three cells that were studied proved to be chromosomally balanced. Since all four of the Pt. Moresby individuals were heterozygous for centric fusions, it is clear that the centric fusions in question must have represented polymorphic variation rather than unique mutants.

The specimens examined from Cape York were found to have 2n = 38 chromosomes including two large metacentrics (Fig. 3.25B). On the basis of their relative arm lengths, these are presumed to represent fusions between chromosomes 1 and 4, and chromosomes 2 and 3 respectively.

This 2n = 38 karyotype with two large metacentrics suggests an ancestral complement of 2n = 42 telocentric chromosomes for this species with the observed karyotypes in the bisexual forms being a

Figure 3.27 (A-B)

C-banded mitotic metaphases of A) the 2n = 39B, and B) the 2n = 28 karyotypes of Nactus arnouxii. Note that C-band heterochromatin is almost exclusively located in centromeric and telomeric regions.


A
B

consequence of progressive reduction in chromosome number by fusion processes. Fusion rather than fusion is regarded by King (1981) as the predominant direction of chromosome change in Gekkonid lizards and its occurrence in N. arnouxii receive some tentative support from the karyotypic data available for related species.

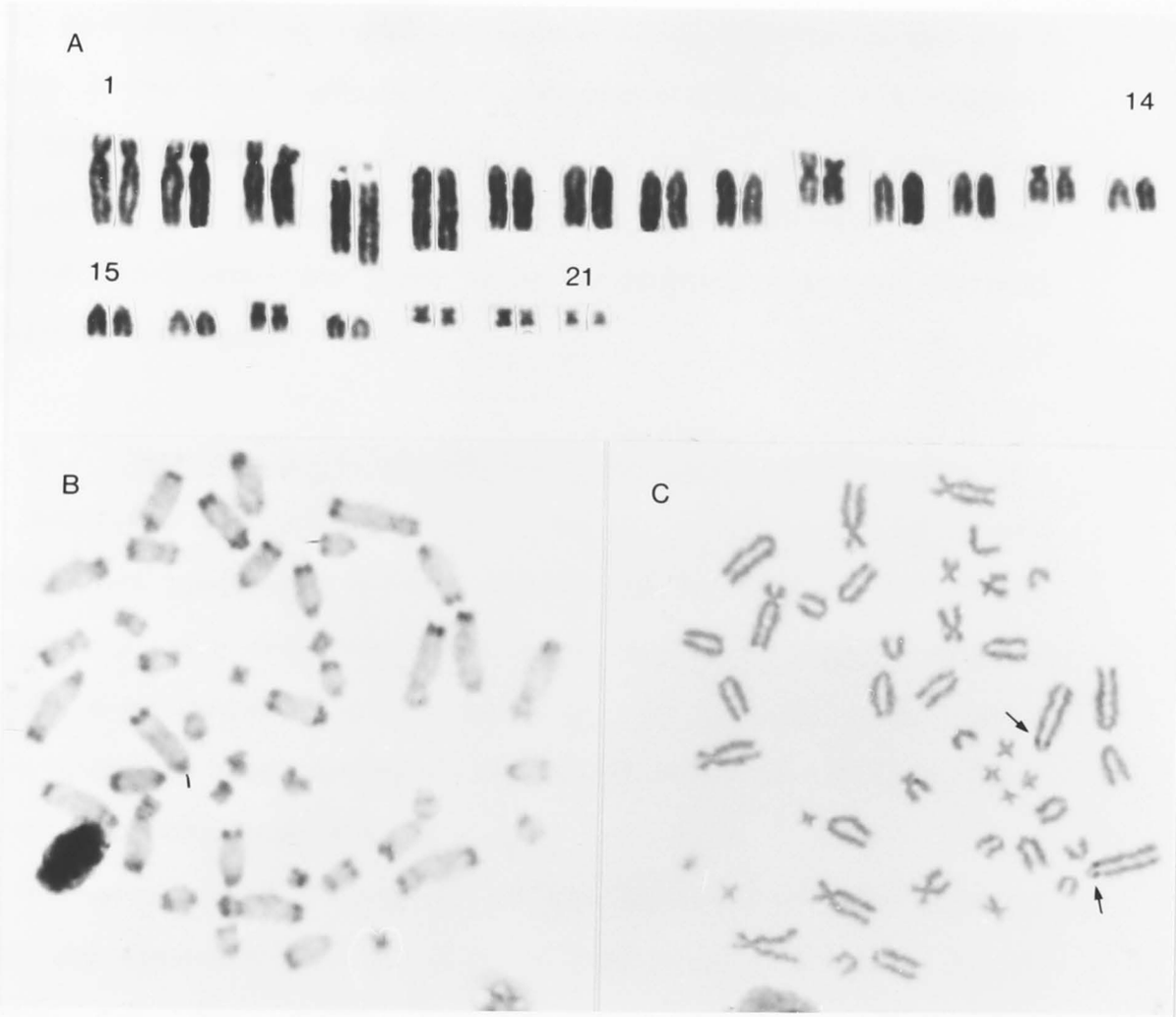
Although no other species of Nactus have been karyotyped to date, two species of Cyrtodactylus (to which N. arnouxii was previously assigned) also have high chromosome numbers. C. lousiadensis has 2n = 42 chromosomes with several submetacentric and metacentric chromosomes (Fig. 3.28A). The short arms of these biarmed chromosomes are not heterochromatic (Fig. 3.27B) and may be a consequence of pericentric rearrangements within an originally telocentric karyotype. C. sp. nov. from Christmas Island (Indian Ocean) also has 2n = 42 chromosomes but has a predominantly telocentric karyotype (King pers. comm.).

If, as the evidence suggests, the primitive karyotype of N. arnouxii consisted of 2n = 42 telocentric chromosomes, then the 2n = 39 karyotypes, each of which had only one metacentric chromosome, must have been derived from the ancestral condition either by a loss of a small pair of telocentric chromosomes or, more probably, by their incorporation into the karyotype by tandem fusion.

The bisexual N. arnouxii from Vanuatu departed radically in karyotype from the other bisexual populations studied. These individuals had 2n = 28 chromosomes of which seven were metacentric or submetacentric and one was acrocentric (Fig. 3.25C). This karyotype had 42 major chromosome arms and can be derived from the proposed ancestral condition by a series of seven Robertsonian fusions. This proposal has been substantiated by C-banding which

Figure 3.28 (A-C).

The karyotype of Cyrtodactylus louisiadensis analysed by A) giemsa staining, B) C-banding and C) silver staining. Note, (i) the short arms of the submetacentric chromosomes are not totally heterochromatic and (ii) the presence of a silver positive secondary constriction (arrowed in C) on the largest pair of telocentric chromosomes.


A
1
14
15
21
B
C

showed that heterochromatin was restricted to centromeric and telomeric regions with the exception of a single interstitial band (Fig. 3.27B). Note also that pair 2 of this karyotype is acrocentric rather than telocentric (cf. Fig. 3.25A and C). Another interesting feature of this karyotype is that the metacentric chromosomes in the first and fifth positions of the karyotype appear to be identical to the 1 + 4 fusion in the Cape York 2n = 38 individuals and the 6 + 8 fusion of the 2n = 39B karyotype respectively. Of course, in the absence of G-banding it is impossible to be definite about the identity of these fusions and reasons are given below for thinking that they are most probably convergent.

(ii) Parthenogenetic populations - distribution and cytogenetics

When the sex ratio data of Schwaner (1980) and Zug (pers. comm.) are considered in conjunction with the analysis of museum specimens, there are three areas of the south-west Pacific that may contain parthenogenetic populations of N. arnouxii (Fig. 3.24). Males were absent in large samples of adults from Samoa (N = 51), Fiji (N = 25) and New Caledonia (N = 16).

Karyotypes of the putative parthenogenetic form were obtained from two specimens from Fiji and one from Niue in the Cook Islands (Table 3.6, Fig. 3.24), and all three proved to be highly heterozygous (Fig. 3.25D). The samples from Niue and Fiji appeared to have identical karyotypes (2n = 2x = 35) with heterozygosity for five Robertsonian fusions and an additional heteromorphism for an acrocentric:telocentric combination of chromosome 2. The arrangement of chromosomes shown in Figure 3.25D is based on arm lengths alone and, once again, G-banding is necessary to conclusively demonstrate

the equivalence of the metacentrics to their proposed telocentric homologues.

The extraordinary heterozygosity of this karyotype immediately suggests parthenogenetic reproduction since, although single fusion heterozygotes may not suffer a serious fitness deficit as a result of meiotic irregularities (see above and also section 1.3), heterozygosity for five fusions is more likely to be significantly deleterious (e.g. Mus musculus, Table 1.2). Moreover, the parthenogenetic populations of N. arnouxii appear to have arisen by hybridisation between karyotypically distinct bisexual populations of this species.

The fusion heterozygosity suggests that the progenitors of the parthenogenetic lineage would have had 2n = 40 and 2n = 30 chromosomes respectively. The homozygous metacentric of the parthenogens (Fig. 3.25D) indicates that the postulated 2n = 40 population was derived from the ancestral 2n = 42 condition by a fusion between chromosomes 7 and 9. A subsequent five fusions and a pericentric rearrangement on chromosome 2 would then account for the postulated 2n = 30 condition. This hypothesis receives strong support from a comparison of the heteromorphisms present in the parthenogen with the karyotype of the 2n = 28 bisexual population (cf. Figs. 3.25C and D). In fact, all of the heteromorphic metacentrics as well as the acrocentric morph of chromosome 2, have structurally identical counterparts in this bisexual race. It is therefore evident that the 2n = 28 race has evolved directly from the proposed 2n = 30 condition by an additional Robertsonian fusion involving chromosomes 11 and 12.

The hypothesised relationships between the bisexual chromosome races and the parthenogenetic biotype of N. arnouxii are summarised

in Figure 3.29. Note that if this scheme is correct, than the 1 + 4 and 6 + 8 fusions that are evidently shared by the 2n = 28 race and the 2n = 38 and 2n = 39B forms respectively are convergent.

(iii) Electrophoretic studies

An electrophoretic study of N. arnouxii was undertaken for two reasons; to test the hybrid origin model proposed on the basis of the cytogenetic studies and to confirm the existance of parthenogenetic reproduction. The sample sizes for each of the bisexual races and the parthenogenetic forms were small (Table 3.7). However, if the number of loci examined is maximised then small samples sizes are expected to have negligible effects on the results if (i) the taxa compared have low heterozygosities, and (ii) they are separated by large genetic distances (Baverstock et al. 1977; Nei 1978; Gorman and Renzi 1979). As will be shown, these requirements are met in this study. The conditions for electrophoresis and the abbreviations used for the loci are defined in section 2.2.

Patterns of allelic variation were determined at 33 presumptive genetic loci from 25 protein systems for each of the bisexual forms with the exception of the 2n = 39B individual for which only 29 loci from 23 protein systems could be scored. Of the 33 loci assayed by electrophoresis, eight did not vary within N. arnouxii (GPT, GOT-2, LDH-B, PGM-1, GP-1, AK, ENO-1 and -2) and the gene frequencies at the remaining 25 loci are given in Table 3.7. Matrices of genetic distances (estimates of Nei (1978) and Rogers (1972)) and percent fixed differences have been calculated from these data and are shown in Table 3.8 and 3.9 respectively.

Figure 3.29

Model of the evolution of the chromosomally distinct forms of Nactus arnouxii. Three distinct lineages are proposed on the basis of the cytogenetic data alone, and in one of these, a series of seven centric fusions have led to the 2n = 28 population from Vanuatu. In this lineage, the hypothetical 2n = 40 and 2n = 30 populations, which hybridized to produce the 2n = 35 parthenogen, are differentiated by a series of five centric fusions involving chromosomes 1 + 4, 3 + 17, 5 + 10, 6 + 9 and 13 + 14. Only the chromosome forms enclosed by boxes have been discovered so far.

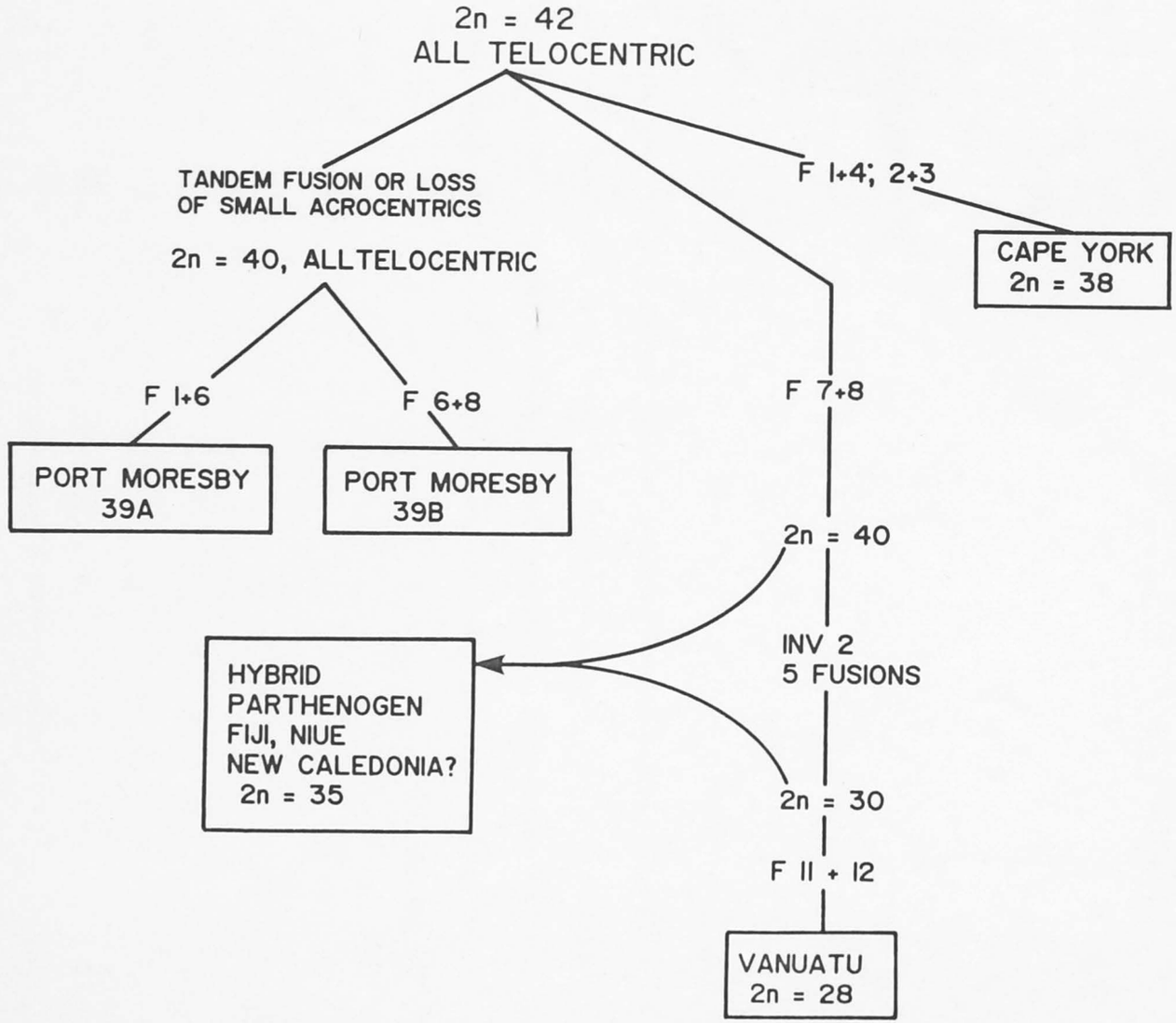

2n = 42
ALL TELOCENTRIC
TANDEM FUSION OR LOSS
OF SMALL ACROCENTRICS
2n = 40, ALLTELOCENTRIC
F 1+6
F 6+8
PORT MORESBY
39A
PORT MORESBY
39B
F 1+4; 2+3
CAPE YORK
2n = 38
F 7+8
2n = 40
INV 2
5 FUSIONS
2n = 30
F 11 + 12
VANUATU
2n = 28
HYBRID
PARTHENOGEN
FIJI, NIUE
NEW CALEDONIA?
2n = 35

Table 3.7

Allele frequencies (expressed as percentages) at the 25 variable loci for Nactus arnouxii. Alleles are designated by their mobility relative to the most common allele of the 2n = 38 form from Cape York. The numbers in brackets are the same sizes for each karyotype.

TABLE 3.7

Locus	Relative Allelic Mobility	BISEXUAL FORMS 2n = 38 (8)	2n = 39A (3)	2n = 39B (1)	PARTHENOGEN 2n = 38 (3)	2n = 35 (7)
Acon-1	1.00	100			100	100
	0.96		100	100		
Acon-2	1.00	100			100	100
	0.81		100	-		
SOD	1.00	100				
	0.63		100			
	0.54				100	100
	0.44			100		
FUM	1.00				100	100
	0.94	100	100	100		
GOT-1	1.20				100	100
	1.13	6				
	1.05		100	100		
	1.00	94				
αGPD	1.00	100			100	100
	0.96		100	100		
ADH-1	1.56		100			
	1.30			-	100	100
	1.00	100				
ADH-2	1.63				100	
	1.00	100	100	-		100
IDH	1.00	82				50
	0.90	18	100	100		
	0.81				100	50
LDH-A	1.00	100	100	100		50
	0.90				100	50
MPI	1.06					
	1.00	100			100	50
	0.94		100	-		50
NDH	1.00	100		50	100	100
	0.95		100	50		
PEP-LP	1.24				100	50
	1.16		33			
	1.08	7		-		
	1.00	56	67			50
	0.95	6				
	0.87	31				
PEP-LGG	1.38			50		
	1.16	12	100	50	100	50
	1.00	69				
	0.96					50
	0.88	19				
6PGD	1.11					50
	1.00	100	100	100	100	
	0.89					50
SorDH	1.20					
	1.14				33	
	1.00	100			67	50
	0.95		84	100		50
	0.91		16			
PGM-2	1.29	14				
	1.09	6				
	1.00	44				
	0.90	6	100			
	0.62	12		100	100	100
	0.48	12				
	0.39	6				
ALD	1.16				100	100
	1.00	100	100	100		
GP-2	1.00	100			100	100
	0.91		100	100		
PK	1.00	100			100	100
	0.90		100	100		
PGI	1.64				17	
	1.00	100	100	100	83	50
	0.72					50
PGK	1.00	100				
	0.91		100	100		
	0.84				100	100
TPI	1.00	100				
	.92		100	100	100	100
HK	1.08				100	
	1.00	100				50
	.91		100	-		
	.86					50
GA3PD	1.00	100				
	0.93		100	100		
	0.88				100	100

Table 3.8

Genetic divergence between the assayed samples of Nactus arnouxii (top) and their heterozygosities. Rogers (1972) genetic distance estimate is above the diagonal and Nei's (1978) genetic distance estimate is below the diagonal.

TABLE 3.8

	SAMPLES				
	1.	2.	3.	4.	5.
N	8	3	1	3	7
1. 38 CAPE YORK		.503	.488	.524	.453
2. 39A PT. MORESBY	.689		.113	.632	.601
3. 39B PT. MORESBY	.651	.096		.537	.557
4. 28 VANUATU	.732	.999	.746		.185
5. 35 FIJI/NIUE	.554	.891	.789	.136	
HETEROZYGOSITY (DIRECT COUNT)	.042	.030	.071	.010	.273

Table 3.9

Genetic divergence between the karyotypes of Nactus arnouxii. Below the diagonal is the per cent fixed differences and above the diagonal is the number of loci used for each comparison.

TABLE 3.9

	SAMPLES				
	1.	2.	3.	4.	5.
N	8	3	1	3	7
1. 38 CAPE YORK		33	29	33	33
2. 39A PT. MORESBY	48.		29	33	33
3. 39B PT. MORESBY	40	7		29	29
4. 28 VANUATU	48	64	48		33
5. 35 FIJI/NIUE	33	55	48	9	

The levels of heterozygosity (direct count method) amongst the bisexual populations are very low, ranging from .01 in the 2n = 28 sample to .042 in the 2n = 38 individuals (Table 3.8). However low levels of heterozygosity are typical for vertebrates in general (Nevo 1978) and geckos in particular (see Table 6.7). In contrast the parthenogenetic individuals, including six specimens from Fiji and one from Niue, had a high heterozygosity of .273 which reflects their fixed heterozygosity at nine of the 33 loci assayed.

With the exception of the 2n = 39A - 39B comparison, the genetic distances between the various cytogenetically defined forms of N. arnouxii are extremely high (Table 3.8). In the former case, the Nei's genetic distance is only .096 (7% fixed differences) so that more individuals of these sympatric forms need to be examined before evaluating Black's suggestion that they represent distinct species. All other comparisons showed that each chromosome form is highly divergent genically (Nei's D range .651 to .999 - Table 3.8; fixed differences range 40 to 64% - Table 3.9).

These values approximate the maximum genetic distances previously documented between congeneric species of reptiles and are twice as high as any intraspecific distances observed in reptiles to date (cf. Tables 3.8 and 6.15). N. arnouxii is also unusual in having large genetic distances between populations that have undergone considerable chromosomal reorganisation as a result of Robertsonian fusions (cf. Tables 3.8, 1.3 and 6.15). Although it is recognised that neither structural gene divergence nor chromosome change have necessarily caused speciation within these taxa (see Chapter 1), the distinctive nature of each of these cytogenetically defined populations indicates that they may warrant specific status.

A morphological study of these populations is in progress to determine if they can be defined as taxonomic species (Moritz and Schwaner, unpublished).

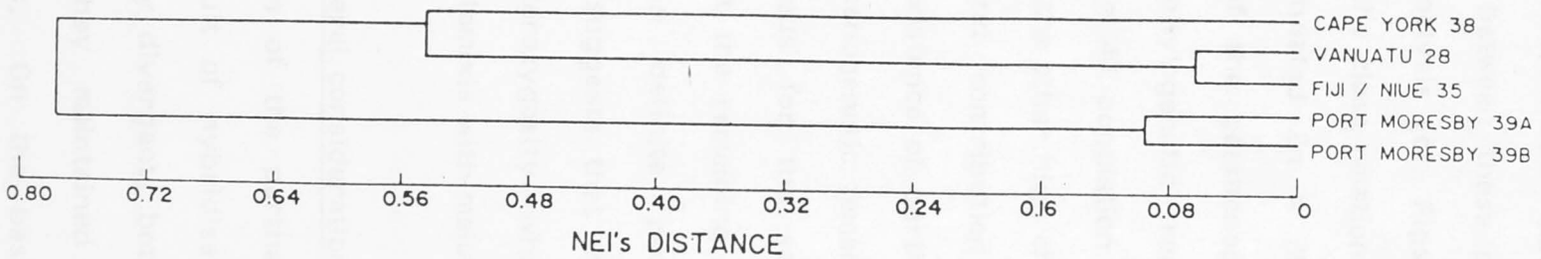
The genic relationships between these bisexual taxa and the parthenogenetic form are especially revealing. Each of the high chromosome number bisexual forms are genically distant from the parthenogenetic clone (Tables 3.8, 3.9). In contrast, the bisexual 2n = 28 form from Vanuatu had only three fixed differences (D_{NEI} = .136) to the 2n = 35 clone (Table 3.9).

These electrophoretic relationships were used to generate an independant phylogeny for these populations of N. arnouxii using the BIOSIS computer package for electrophoretic statistics (see section 2.2). Two approaches were adopted; (i) a dendogram based on Nei's genetic distance (Fig. 3.30A) and (ii) a mid-point rooted distance - Wagner tree generated using Rogers distance estimate (Fig. 3.30B). The relative merits of these approaches are briefly discussed in section 6.4.2 and are of no concern here. The phylogenetic trees show in Figure 3.30 are based on only 29 loci to allow the inclusion of the 2n = 39B individual. When this animal was excluded, and all 33 loci were analysed, the dendogram was not altered but as a consequence of a shift in the midpoint of the Wagner network, the Cape York sample was now grouped (but only just) with the 2n = 39A population rather than the 2n = 28 + 35 group as before. For this reason, the phylogeny of these populations in best viewed as three lineages that emerge from an unresolved trichotomy; (i) the 2n = 38 sample (ii) the 2n = 39A and B populations and (iii) the 2n = 28 population together with the parthenogenetic clone (Fig. 3.30B).

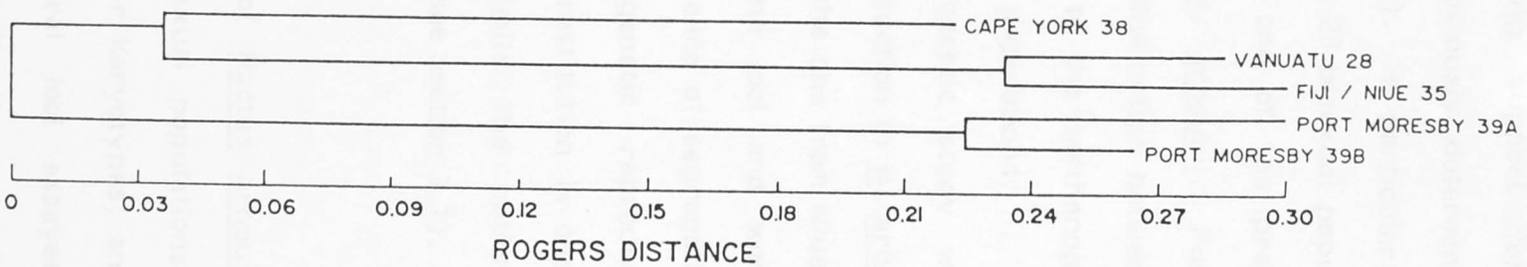
Figure 3.30 (A-B)

Electrophoretic relationships between the chromosomally distinct populations of Nactus arnouxii, presented as A) a dendogram based on Nei's (1978) genetic distances and B) an unrooted distance - Wagner network calculated from Rogers' (1972) genetic distances. In these analyses, only the 29 loci for which data is available in all of the chromosome forms have been included. The small genetic distance between the Cape York 2n = 38 Vanuatu 2n = 28; Fiji and Nive 2n = 35 branch is not significant (see text). The cophenetic correlations of the trees in A and B are 0.96 and 1.00 respectively.

A


CAPE YORK 38
VANUATU 28
FIJI / NIUE 35
PORT MORESBY 39A
PORT MORESBY 39B
0.80
0.72
0.64
0.56
0.48
0.40
0.32
0.24
0.16
0.08
0
NEI's DISTANCE

B


CAPE YORK 38
VANUATU 28
FIJI / NIUE 35
PORT MORESBY 39A
PORT MORESBY 39B
0
0.03
0.06
0.09
0.12
0.15
0.18
0.21
0.24
0.27
0.30
ROGERS DISTANCE

The electrophoretic data provide strong support for the relationships between these populations as previously determined by cytogenetic analysis (cf. Figs. 3.30B and 3.29). In particular, they substantiate the close relationship between 2n = 28 bisexual population and the postulated 2n = 30 form that was one of the presumed progenitors of the parthenogenetic form of N. arnouxii. Further, both of the phylogenetic trees highlight the distinction between the proposed 2n = 40 population that contributed to the parthenogenetic genotype and the other high chromosome number populations.

The second contribution of the electrophoretic study was to confirm the existence of parthenogenetic reproduction in N. arnouxii. The six parthenogenetic females from Fiji and the one from Niue were all heterozygous for the same alleles at nine loci and were all homozygous at the remaining 24 loci. The absence of segregation at these loci is definite proof of parthenogenetic reproduction. Moreover, it suggests that the mechanism of restitution is one that maintains heterozygosity which effectively limits the options to apomixis or automixis with meiotic reduplication (see section 3.1).

(iv) General considerations

The origin of the parthenogenetic clone of Nactus arnouxii is clearly a result of hybridisation between bisexual populations that were extremely divergent, both in terms of their karyotypes, and for the alleles they maintained at the structural loci assayed by electrophoresis. On the basis of what is admittedly very limited sampling, there appears to be only a single clone of the parthenogen. However, it must be noted that no representatives of the presumed parthenogenetic populations from New Caledonia have been studied

and sample sizes in other areas need be increased before this homogeneity can be considered as a general feature of this parthenogen.

With regard to genetic diversification within the parthenogenetic lineage, the cytogenetic evidence do not indicate any heteromorphisms beyond those expected as a result of the hybrid origin. However, it is difficult to make the same claim for the electrophoretic heterozygosity observed in the parthenogens; many of the alleles have not been identified from the bisexual samples studied and conclusive evidence on this matter must await further sampling.

Beyond the fact that parthenogenesis in N. arnouxii has become established in a tropical environment, little can be said about the comparative zoogeography of the bisexual and subsexual forms of this species. The available evidence indicates that the bisexual populations are predominant in the western portion of the species distribution (Fig. 3.24) with the parthenogens occupying the remainder of the area so far sampled. It is probable that the evolution of parthenogenesis has facilitated the colonisation of the islands to the south and east of Vanuatu and on this basis, it would be predicted that N. arnouxii at the extreme eastern limit of its distribution (Tuamoto Archipelago) would also be parthenogenetic.

3.2.3. Lepidodactylus lugubris

The genus Lepidodactylus consists of three groups defined on morphological criteria; two in Australia and Melanesia and a third which includes four species from the Philippines in addition to the widespread lugubris - woodfordi complex (Brown and Parker 1977).

L. lugubris extends throughout the tropical region between India and central America and is found on the intervening landmasses such as Papua New Guinea, Cape York and the Pacific islands (Brown and Parker loc. cit.). Within this distribution, this species is highly commensal with man and it is also found in natural habitats although usually at lower densities (Pernetta and Black 1983).

Parthenogenesis in L. lugubris was recognised by Cuellar and Kluge (1972) on the basis of the general rarity of males, the low level of tissue homograft rejection and the absence of sperm in ovulating females. In 10 females from Oahu (Hawaii) that were examined karyotypically, these authors obtained counts of 44 chromosomes, most of which appeared to be telocentric. However, this situation was complicated by the fact that Gehyra variegata ogasawarasimae, regarded by Hall (1970) as a possible parthenogenetic species was subsequently synonymised with L. lugubris (Shigei 1971 in Kluge 1982). A female of this population, obtained from the Ogasawara islands off Japan, was found by Makino and Momma (1949) to have 63 rod shaped chromosomes in each of the 4 oogonial spreads examined which was interpreted by Hall (1970) as evidence for triploidy. In a brief review of these observations, Kluge (1982) stressed the need for further karyotypic studies on L. lugubris.

(i) Cytogenetic studies

Karyotypes were obtained for 24 female L. lugubris from islands of the central and western Pacific (Fig. 3.31) and seven of these individuals were C-banded (Table 3.10).

Amongst the animals examined, two ploidy levels were found, 2n = 2x = 44 and 2n = 3x = 66. In agreement with Cuellar and Kluge

Table 3.10

Localities from which Lepidodactylus lugubris were karyotyped and the results obtained for each sample. The figures in parentheses are the number of individuals C-banded. The locality numbers refer to points on figure 3.31.

TABLE 3.10

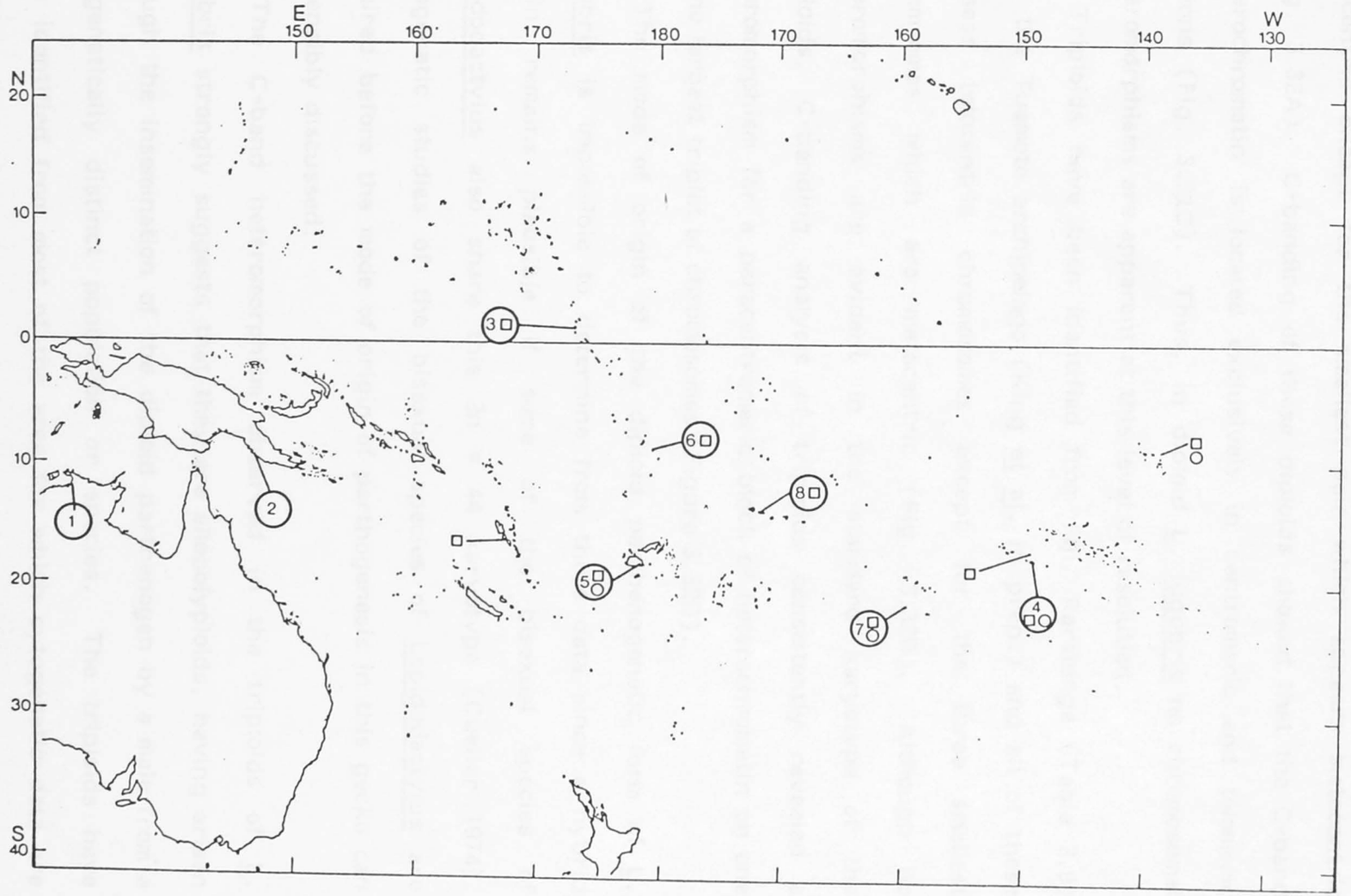
LOCALITY	PLOIDY	
	2n = 2x = 44	2n = 3x = 66
4. TAHITI	1	
5. FIJI	13 (2)	2 (2)
3. KIRIBATI	1 (1)	
6. TUVALU	1	
7. RARATONGA	3 (1)	2 (1)
8. WEST SAMOA	1	

Figure 3.31

Map of the islands of the western Pacific Ocean showing the geographic location of the samples of Lepidodactylus lugubris and Hemidactylus frenatus. The numbers refer to Tables 3.10 and 3.11. The results of cytogenetic analyses of L. lugubris from the Tuamoto archipelago and Tahiti (King et al. in prep.) are also included.

Symbols: □ diploid L. lugubris

○ triploid L. lugubris


E
150
160
170
180
170
160
150
140
W
130
N
20
10
0
10
20
30
S
40
1
2
3
4
5
6
7
8

(1972) the diploid has 22 pairs of chromosomes which are all telocentric except for the smallest pair which appears metacentric (Fig. 3.32A). C-banding of these diploids showed that the C-band heterochromatin is located exclusively in centromeric and telomeric regions (Fig. 3.32C). Thus, in diploid L. lugubris no chromosomal heteromorphisms are apparent at this level of resolution.

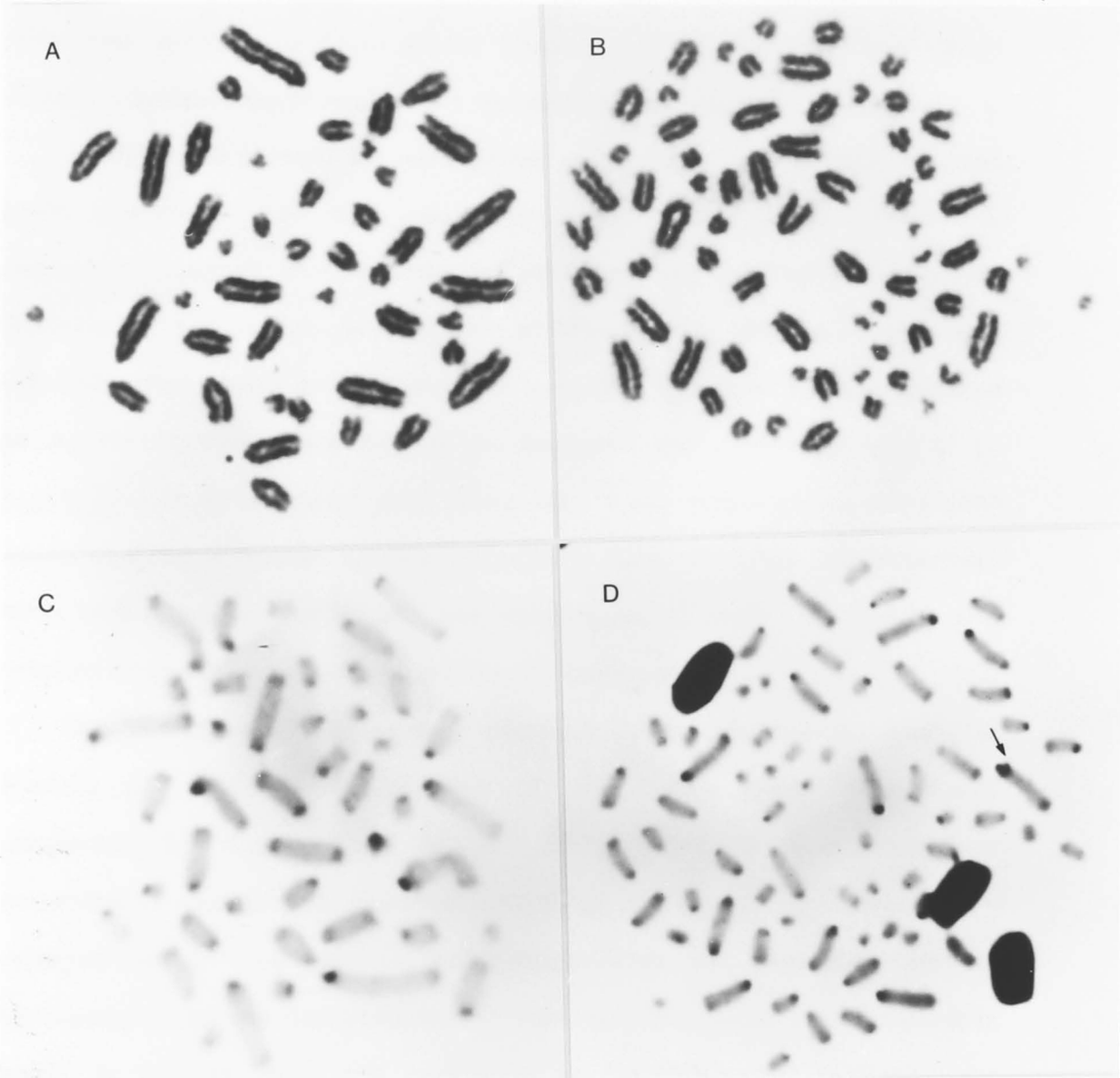
Triploids have been identified from Fiji, Raratonga (Table 3.9) and the Tuamoto archipelago (King et al. in prep.) and all of these possess telocentric chromosomes except for the three smallest chromsomes which are metacentric (Fig. 3.32B). Although no heteromorphisms are evident in the standard karyotype of the triploids, C-banding analysis of triploids consistently revealed a heteromorphism for a paracentromeric block of heterochromatin on one of the largest triplet of chromosomes (Figure 3.32D).

The mode of origin of the diploid parthenogenetic form of L. lugubris is impossible to determine from these data since a hybrid origin remains plausible if some of the bisexual species of Lepidodactylus also share this 2n = 44 karyotype (Cuellar 1974). Cytogenetic studies of the bisexual species of Lepidodactylus are required before the mode of origin of parthogenesis in this gecko can be sensibly discussed.

The C-band heteromorphism observed in the triploids of L. lugubris strongly suggests that they are allopolyploids, having arisen through the insemination of the diploid parthenogen by a male from a cytogenetically distinct population or species. The triploids have been identified from most of the area for which cytogenetic data are available (Fig. 3.31) so that it cannot be determined where they arose. While bisexual species of Lepidodactylus are evidently absent

Figure 3.32 (A-D)

Karyotypes of Lepidodactylus lugubris. A) Giemsa stained karyotype of a diploid, 2n = 2x = 44. B). Giemsa stained karyotype of a triploid, 2n = 3x = 66. C). C-banded cell of a diploid. Note that C-band heterochromatin is restricted to centromeric and telomeric regions. D) C-banded cell of a triploid. Note the heteromorphic paracentromeric C-band on one large telocentric chromosome (arrowed).


A
B
C
D

from the south-central Pacific, it remains possible that the triploids arose in this region following insemination of the diploids by locally common male L. lugubris (see below). This hypothesis is consistent with the absence of phenotypic divergence in the triploids which could reasonably be expected if they were interspecific hybrids.

The demonstration of widespread triploidy in L. lugubris does lend credence to the suggestion by Hall (1970) that the representatives of this species in the Japanese islands are triploid. However if the chromosome counts of Makino and Momma (1949) were accurate then these populations ($2n = 3x-3 = 63$) must have originated as a cross between the diploid parthenogen and a $2n = 38$ form of L. lugubris. Alternatively, this clone may have acquired its divergent karyotype as a result of the secondary loss of three chromosomes. With this possible exception, the karyotypes of both the diploid and triploid clones of L. lugubris are highly conserved.

The existance of widespread allotriploids in this species may also explain an aberrant observation of the tissue graft experiments conducted by Cuellar and Kluge (1972). Of the nine transplant experiments conducted between Hawaiian L. lugubris, three were rejected which was not in accordance with the expected genetic homogeneity across individuals. These authors went to considerable pains to explain away the rejections as methodological artifacts but were forced to conclude that some of the Hawaiian populations of L. lugubris may have been genetically divergent. A simple and plausible explanation for the occasional graft rejections is that they involve transplants between diploids and allotriploids.

A particularly interesting feature of L. lugubris is the occasional presence of males. In general these are very rare (Solomon Islands

3/217; Hawaii - 1/221, Cueller and Kluge 1972) but on one island in the Tuamoto archipelago, 12 of the 30 L. lugubris examined were males. (King et al. in prep.). It is conceivable that the rare males could be the result of hormonal sex inversion or disturbances in the balance of the sex determining system as suggested by Darevsky et al. (1978) to account for a similar phenomenon in parthenogenetic Lacerta. However it is unlikely that either of these process could be responsible for the locally abundant males of the Tuamoto Archipelago. A very real possibility is that these individuals represent a bisexual sibling species to L. lugubris. A detailed cytogenetic and electrophoretic study of this population is of considerable importance to further understanding of both the origin of diploid parthenogenesis in L. lugubris and the evolution of the allotriploid clone.

3.2.4 Hemidactylus

Hemidactylus, which is an extremely widespread and taxonomically diverse genus, includes two species that are considered to be parthenogenetic. H. garnotii is an all-female species which is widely distributed in the tropical zone between south-east Asia to the west and Florida (North America) to the east (Kluge and Eckardt, 1969). Recently Darevsky et al. (in press) have identified another, morphologically divergent parthenogenetic species; H. vietnamensis and this species has been recorded from localities in China, Burma, India and Indo-China.

Kluge and Eckardt (loc. cit.) karyotyped specimens of H. garnotii from Florida and Hawaii and found that these species had a modal number of 2n = 70 chromosomes which is suggestive of

triploidy. Further, these karyotypes were invariably heteromorphic for one chromosome triplet (with three distinct chromosome morphologies - see Fig. 3 in Kluge and Eckardt 1969) and expressed secondary constrictions on only two of the three largest telocentric chromosomes.

The standard karyotype of H. vietnamensis which was studied by Darevsky et al. (in press) is distinctive from that of H. garnotii since it has only 60 chromosomes. While this species also appears to be a triploid, the only observed heteromorphism involved secondary constrictions. As was the case for H. garnotii, these were only expressed on two of the three largest telocentric chromosomes. In view of the dosage compensation observed for NOR activity in triploid Heteronotia (see section 3.2.1-ii), the secondary constriction heteromorphisms observed in both of the triploid species of Hemidactylus may simply represent random inactivation of one set of ribosomal genes rather than a true structural polymorphism.

The structural chromosomal heteromorphisms present in H. garnotii suggest a hybrid origin of this parthenogen. However detailed cytogenetic studies of the numerous bisexual species of Hemidactylus are required to test this hypothesis. The six bisexual species of this genus that have been karyotyped todate range in chromosome number from 2n = 40 to 2n = 46 (reviewed by King 1978). The widespread H. frenatus is characterised by 2n = 40 chromosomes in Australia (King, 1978) and Vietnam (Darevsky et al., in press) however Makino and Momma (1949) reported a diploid number of 46 chromosomes for this species in Formosa.

H. frenatus were karyotyped from three areas in the western Pacific and, with one exception, were found to have karyotypes that

Table 3.11

Localities from which Hemidactylus frenatus were karyotyped and the results obtained for each sample. The locality numbers refer to points on figure 3.31.

TABLE 3.11

	LOCALITY	N	CHROMOSOME NUMBER
1.	DARWIN, AUSTRALIA	2	40
2.	PT. MORESBY, PAPUA NEW GUINEA	2	40
3.	TARAWA, KIRIBATI	2	40,60

Figure 3.33 (A-D)

Karyotypes of Hemidactylus frenatus. A). Giemsa stained karyotype of a diploid, 2n = 2x = 40. B) Giemsa stained karyotype of the single individual that was found to be triploid (2n = 3x = 60). The arrow-head indicates a secondary constriction that was the only heteromorphism observed in the triploid karyotype. C-D). C-banded cells of diploid and triploid H. frenatus respectively. Note that C-band heterochromatin is restricted to centric and telomeric regions.

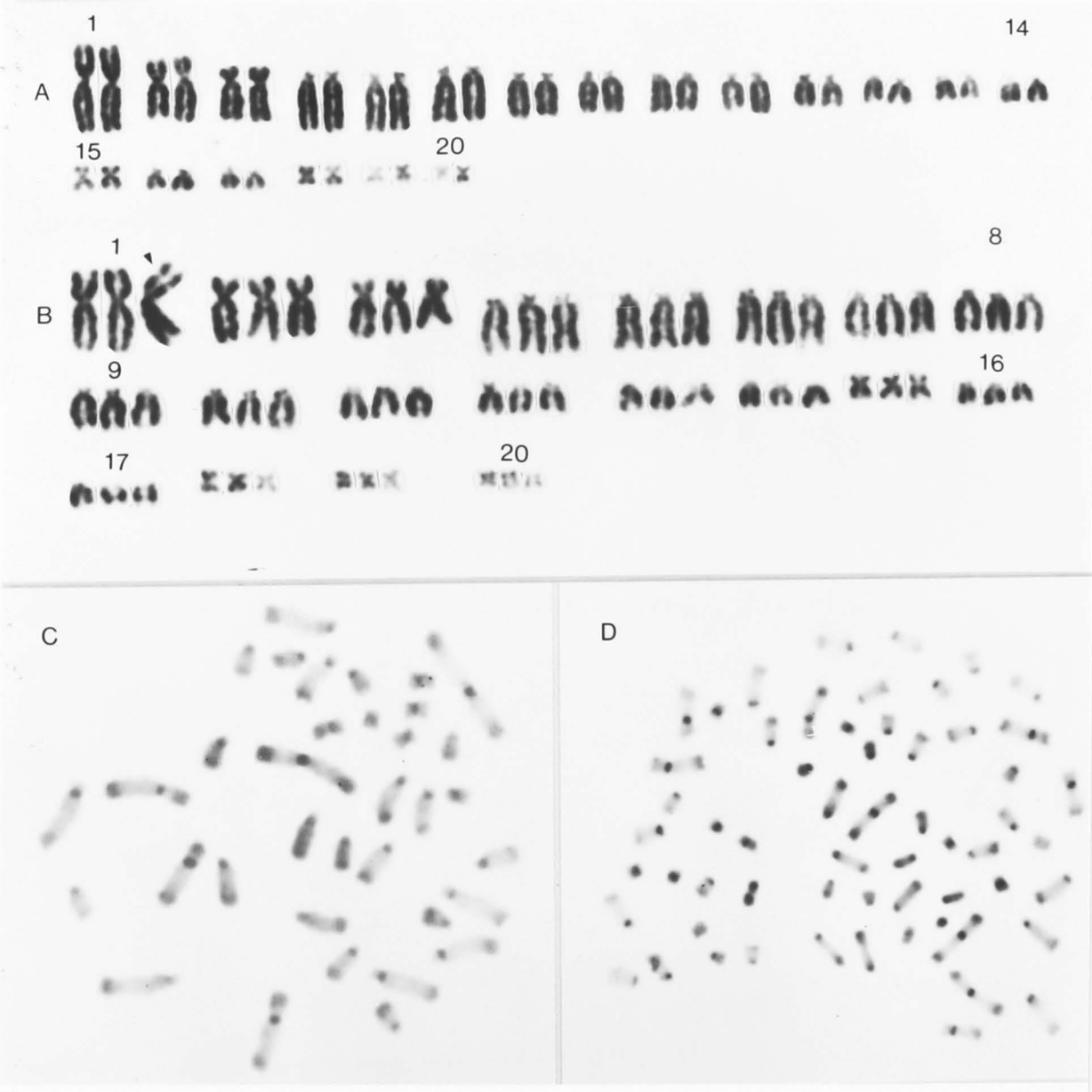
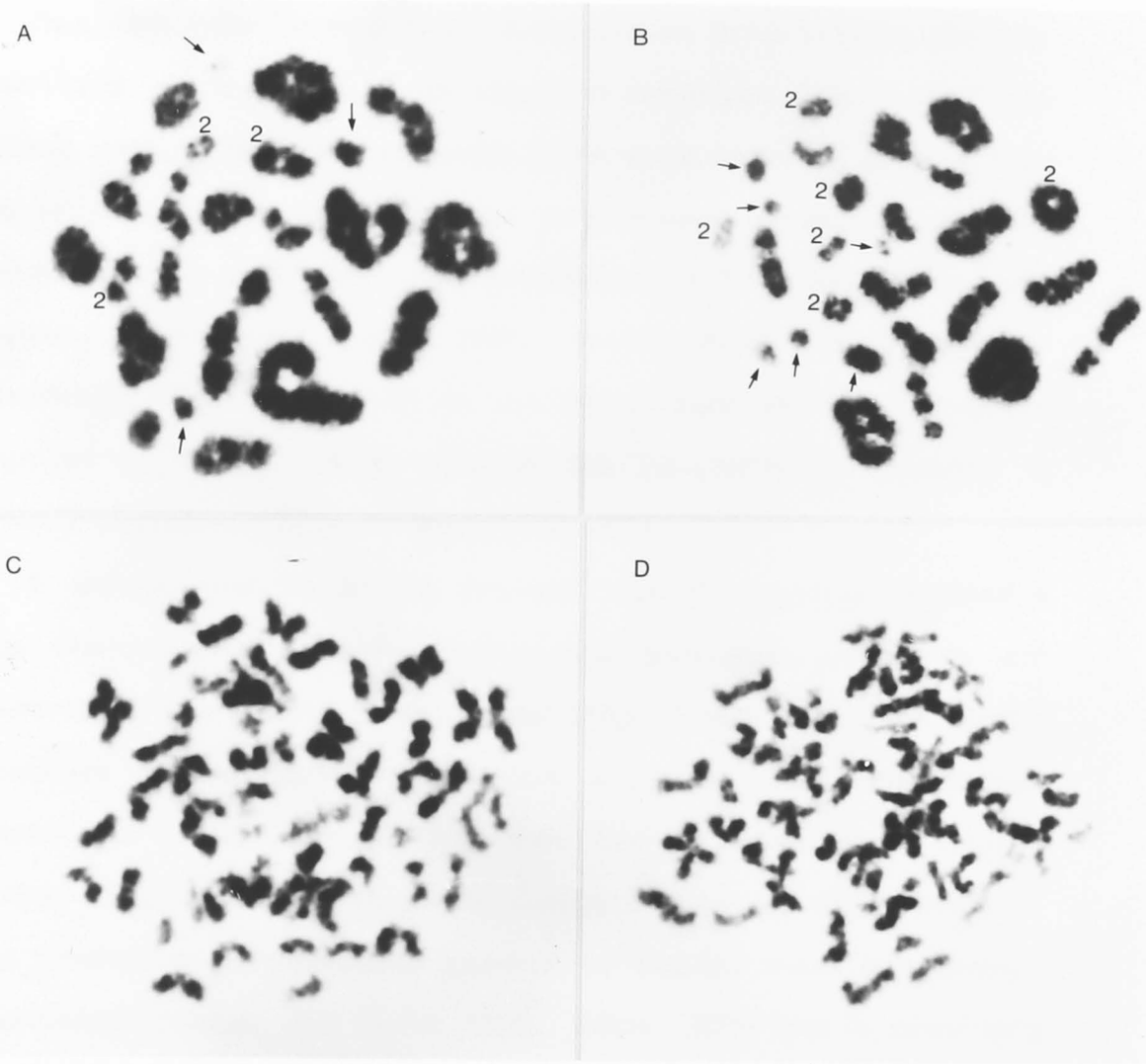

A
1
14
15
20
B
1
8
9
16
17
20
C
D

Figure 3.34 (A-D)

Meiotic metaphases from the triploid Hemidactylus frenatus A-B). First metaphases (or possibly diakinesis) showing the high frequency of univalents (arrowed) and bivalents. The remaining associations are interpreted as trivalents. C-D). Second meiotic metaphases from the same individual. Although precise counts of dyads are difficult to obtain, these cells appear to have approximately the triploid (60) number of dyads which indicates a failure of first anaphase.


A
2
2
2
B
2
2
2
2
2
2
C
D

were morphologically identical to the previously reported 2n = 40 complement (Table 3.11, Fig. 3.33A). C-banding of 2n = 40 individuals from each of these localities revealed centromeric and telomeric blocks of heterochromatin and an absence of interstitial bands (Fig. 3.33C).

One male from Kiribati (Fig. 3.31) proved to be triploid (2n = 3x = 60) and, on the basis of its standard karyotype (Fig. 3.33B) and C-band pattern (3.33D), appeared to be an autotriploid derived from the sympatric 2n = 40 population. Spontaneous triploidy in bisexual vertebrates has previously been documented in fish, amphibians, and reptiles (Cuellar and Uyeno 1972; Witten 1978) and is generally considered to be the result of the insemination of rare unreduced eggs by a haploid sperm although the possibility of dispermy is difficult to disprove (Cuellar and Uyeno 1972).

A meiotic analysis of the triploid male H. frenatus revealed a high frequency of first metaphase cells with some univalents and bivalents in the place of trivalents (Fig. 3.34A, B). The second metaphase spreads obtained for this individual were not of high quality but it was evident that they had approximately a triploid number (Fig. 3.34 C, D) which suggests a failure of anaphase I. The production of unreduced gametes by triploid males is a common phenomenon (Becak and Becak 1970; Witten 1978) and is possibly a direct mechanical result of univalents lagging at anaphase. This, in turn, led to a failure of cytokinesis; a process that has been demonstrated in some orthopterans (reviewed by White 1973). The ability of these triploid gametes to produce functional sperm is not known although Becak and Becak (1970) suggested that spontaneous triploidy may be a step towards the production of viable bisexual tetraploids.

3.3. The Maintenance of Sex in Vertebrates

Over 30 subsexual species of diploid or triploid vertebrates have been discovered to date (Table 3.12 and 3.13 and see Bell 1982). The predominant modes of subsexuality amongst fishes and amphibians are sperm dependant; gynogenesis has evolved in five genera and hybridogenesis in two others. In these cases sperm are required for the initiation of development (gynogenesis) or are inolved in fertilisation but are then discarded at meiosis (hybridogenesis) and these taxa are therefore regarded as reproductive parasites on their bisexual species which provide sperm (Table 3.1). The only suggestion of true parthenogenesis in these classes comes from the apparent absence of males from some large population samples of the salamander Ambystoma (Downs 1978) and the absence of sperm in some ovulating females of the same species (Cuellar 1976). By contrast the only mode of subsexual reproduction found so far in reptiles is parthenogenesis (Table 3.12).

Four areas of empirical research are necessitated by the theoretical models reviewed in section 3.1, namely (i) the origin of the genetic mechanisms responsible for parthenogenesis, (ii) the extent of genetic diversity within unisexual lineages, (iii) the environmental correlates of parthenogenesis and (iv) the comparative niche breadth and reproductive performance of bisexual and subsexual populations. The first of these is critical to the historical hypothesis for the maintenance of sex (see section 3.1) while the remaining areas of research can provide valuable information on the selective forces that operate on subsexual populations once they have arisen and become established. Evidence from studies of vertebrates (and invertebrates were relevant) that is pertinent to these questions is considered below.

Table 3.12

Summary of the subsexual reproductive mechanisms found amongst fishes, amphibians and reptiles. Abbreviations: under reproduction - G = gynogenesis, H = hybridogensis, P = parthenogenesis; under restitution - A = apomictic (no restitution required), M = meiotic reduplication, 1 = intrameiotic fusion; under origin - H = hybrid origin, S = spontaneous. Note, (1) some populations of Ambystoma may be parthenogenetic, (2) Menidia is thought to a diploid but this claim has not been substantiated by cytogenetic studies, (3) the parthenogenetic Leiolepsis is not regarded by Peters (in Cole 1975) as an interspecific hybrid and the same argument has been applied to Lepidophyma flavimaculatum.

TABLE 3.12

TAXA	REPRO-DUCTION	PLOIDY	RESTI-TUTION	ORIGIN	REFERENCES
FISH					
Poeciliidae: Poecilia	G	2X/3X	A	H	Turner 1982
: Poeciliopsis	H	2X	A	H	Schultz 1977; Cimino 1972a
: Poeciliopsis	G	3X	M	H	Schultz 1977; Cimino 1972b
Atherinidae: Menidia	G	2X?		H	Echelle et al. 1983
Cyprinidae: Carassius	G	2X/3X	I	S	reviewed in Schultz 1977.
AMPHIBIANS					
Ranidae: Rana	H	2X/3X	M	H	Berger 1983, Heppich et al. 1982
Ambystomidae: Ambystoma	G(P?)	2X/3X	M	H	MacGregor & Uzzell 1964; Downs 1978
REPTILES					
Agamidae: Leiolepis	P	3X		S?	Hall 1970, Peters in Cole 1975
Chameleonidae: Brooksia	P			?	" "
Gekkonidae: Lepidodactylus	P	2X/3X		H	Section 3.2.3
: Hernidactylus	P	3X		?	Section 3.2.4
: Heteronotia	P			H	Section 3.2.1
: Nactus	P	2X		H	Section 3.2.2
Lacertidae: Lacerta	P	2x		H	Uzzell & Darevsky 1975
Teiidae: Cnemidophorus	P	2X/3X	M	H/S	see Table 3.13
: Gymnophthalmus	P		?	?	reviewed in Cole 1975
Xantusiidae: Lepidophyma	P	2X		S	Bezy 1972
Typhlopidae: Typhlina	P	3X?		?	McDowell 1974; Mengden pers. comm.

3.3.1 The origin of subsexual reproduction in vertebrates The vast majority of unisexual vertebrates that have been studied to date present either the morphological, electrophoretic or cytogenetic characteristics expected of hybrids between genetically divergent taxa (Table 3.12 and 3.13). Approximately half of the species studied have proved to be triploid and it is generally agreed that these have arisen as a result of backcrossing between diploid parthenogens and genetically distinct bisexual taxa (Cuellar 1978; White 1978a; Bell 1982). However the origin of diploid subsexuality, and thus the primary evolution of these reproductive modes, remains a contentious issue.

Early workers (e.g. Uzzell 1970) considered that virtually all diploid vertebrate subsexuals may have arisen as a consequence of interspecific hybridisation. However Cuellar (1974) pointed out several deficiencies in the hybrid-origin model as it was proposed at that time and most authorities in this field now accept a duality of modes of origin of subsexual vertebrates; a hybrid origin or its evolution through tychoparthenogenesis (e.g. Cole 1978; Wright 1978; White 1978a).

Cuellar (1974, 1977, 1978) has been the principal proponent of the view that subsexual reproduction (specifically parthenogenesis) may arise spontaneously within a bisexual population as is the case in many apomictic and automictic invertebrates with intra- or post meiotic restitution (Bell 1982). Cuellar (1974) cited several examples of tychoparthenogenesis in both invertebrates and vertebrates in support of his model. However, it should be noted that all of these cases restore the somatic poloidy level by way of intra- or post meiotic restitution which is distinct to the predominant meiotic mechanisms observed in subsexual vertebrates (see below and Table 3.12).

Table 3.13.

Review of the data on the cytogenetics and origin of the parthenogenetic species of Cnemidophorus. The chromosome formula used follows Lowe and Wright 1966. Note, (1) in some of the early cytogenetic studies in particular, the number of microchromosomes may not always be reliable. The deletion of a major chromosome arm in C. cozumela was reported by Fritts (1969) but was not verified by Lowe et al. 1970b. (2) In many cases a hybrid origin has been confirmed electrophoretically but the only extensive study of electrophoretic variation is for C. tesselatus (Parker and Selander 1976). (3) Modern chromosome banding techniques have only been applied to a single specimen of C. laredoensis (Bickham et al. 1976) and several C. tigris; a bisexual species (Bull, 1978).

TABLE 3.13

Species	Ploidy	2N	Chromosome formula	Proposed origin**	Cytogenetic modifications	References
C. laredoensis	2X	46	2+24+20	*+ C. gularis x C. sexlineatus		McKinney et al. 1973 Bickham et al. 1976 Wright et al. 1983
C. lemniscatus	2X	50 48 48	2+24+24 2+24+22 2+24+22	Spontaneous or intraspecific hybrid	Loss of pair of small acrocentrics, inversions	Peccinini-Seale and Frota-Pessoa 1974
C. cozumela	2X	49 47 47	0+28+21 1+26+20 0+27+20	C. deppei x C. augusticeps	Deletion of major chromosome arm	Lowe et al. 1970b Fritts 1969
C. neomexicanus	2X	46	4+20+22	*+ C. tigris x C. inornatus		Lowe & Wright 1966 Neaves 1969 Brown & Wright 1979
C. tesselatus++	ca.2X ca.3X	46 69	4+20+22 5+32+32	*+ C. tigris x C. septemvittatus 2X x C. sexlineatus		Lowe et al. 1970b Neaves 1969 Parker & Selander 1976 Brown & Wright 1979
C. uniparens	3X	69	3+36+30	+C. gularis (?) x C. inornatus x C. inornatus		Lowe & Wright 1966 Neaves 1969
C. exsanguis	3X	69 70 71	3+36+30 2+37+31 2+37+32	+ C. gularis x C. inornatus X S	Centric fission, 'extra' small acrocentrics	Neaves 1969 Cole 1979
C. sonorae	3X	69 70 71	2+38+29 2+38+30 1+40+30	S x S x S	centric fission, extra small acrocentrics	Lowe et al. 1970a
C. velox	3X	68	3+34+31	+C. inornatus x C. inornatus x S		Pennock 1965 Neaves 1969

\+ Confirmed by electrophoretic studies
++ Modified karyotypes exist in 2x and 3x forms but no details given (Lowe et al. 1970b)
** S indicates an unidentified member of the sexlineatus group (see Lowe et al. 1970b)
* Maternal ancestor, as determined by mitochondrial DNA analysis, is indicated first.

The widely accepted hybrid origin model, which was subjected to critical evaluation by Cuellar (1974), was succinctly summarised by Cole (1975). Interspecific hybridisation occurs in an ecotone between two ecologically distinct bisexual species and results in somatically vigorous but largely sterile hybrids. Rare hybrids are endowed with a specific gene combination that allows for the production of diploid ova that can subsequently undergo development. This mechanism provides an escape from sterility, commonly presumed to be the consequence of extensive asynapsis or other mechanical meiotic irregularities that occur in an allodiploid (Uzzel and Goldblatt 1967; Sessions 1982). The newly established parthenogenetic lineage must subsequently avoid insemination by conspecific males in order to prevent extinction through the production of sterile backcrosses of higher ploidy levels (Cuellar 1977).

The most significant and widely accepted objection to the origin of subsexual reproduction by hybridisation is the absence of a direct link between the required meiotic mechanisms and the effects of hybridisation per se (Cuellar 1974, 1978; Cole 1978; Wright 1978; White 1978a). Indeed Maynard-Smith (1978 p. 48) stated that "hybridisation is not by itself sufficient to cause parthenogenetic development". Clearly, the development of a comprehensive and realistic theory for the evolution of subsexuality in vertebrates requires a consideration of the genetic control of the meiotic mechanisms employed by these taxa.

This aspect has been studied primarily in subsexual fishes and amphibians (Table 3.12), in which the examination of female meiosis is facilitated by relatively high levels of fecundity. Two of the species studied so far appear to be apomictic (gynogenetic Poecilia; Turner

1982, and hybridogenetic Poeciliopsis; Cimino 1972a). Only one of the automictic species has a restitution mechanism other than a meiotic reduplication; this is the goldfish Carassius which has an abortive first division although Shultz (1977) questioned whether gynogenesis in this species arose naturally or was the result of the extensive and long term genetic manipulations by man.

The only conclusive study of female meiosis in a parthenogenetic reptile concerns the Teiid lizard Cnemidophorus uniparens. Cuellar (1971) demonstrated that the somatic ploidy level of this species is retained by way of an additional meiotic DNA replication. In other subsexual vertebrates the restitution mechanisms has been inferred from the apparent rarity of recombination between genetic markers in the subsexual diploids (Uzzell 1970; Vrijenhoek et al. 1978). This observation, which also appears to be the case for Nactus arnouxii (section 3.2.2), led Uzzell (loc. cit.) to propose that meiotic reduplication may be the predominant mechanism in subsexual vertebrates. Although apomixis cannot be excluded, the former mechanism is also compatible with the evident absence of recombination and triploidy in Heteronotia binoei (section 3.2.1.- iii). Meiotic reduplication also occurs in all obligate parthenogenetic orthopterans (Scali 1982) but has not been demonstrated in any examples of tychoparthenogenesis. This detracts from Cuellar's (1974) proposition that vertebrate parthenogenesis arose via tychoparthenogenesis.

With few exceptions, meiotic reduplication has been demonstrated by analysis of diplotene and the subsequent stage of meiosis but it is important to restate that the exact timing of the extra DNA replication is not clear. It has generally been assumed (Uzzell 1970; Cuellar 1971, 1976; MacGregor and Uzzell 1964) that this process

occurs during the last premeiotic mitosis. However, in a recent study of gynogenetic Ambystoma, Sessions (1982) provided evidence for a pachytene reduplication. The details of this mechanism have been established in a series of studies on parthenogenetic stick insects (Koch et al. 1972; Pijnacker and Ferwarda 1978, 1982). Primary synapsis initially occurs between homologous chromosomes but the synaptonemal complex is degraded during the pachytene reduplication after which secondary synapsis almost invariably occurs between the identical sister chromosomes. Chiasmata are only formed after the reduplication and at diplotene the somatic number of bivalents are observed. The general restriction of recombination to the molecularly identical sister chromosomes has been confirmed cytologically by White et al. (1963) in Warramaba virgo although chiasma formation between homologous (i.e. non-sister) chromosomes has been observed at low frequency (0.1 to 1.0%) in the stick insect Carasius morosus (Pijnacker and Ferwarda 1982) and Ambystoma (MacGregor and Uzzell 1964) and is suggested by the electrophoretic study of Cnemidophorus tesselatus (Parker and Selander 1976).

The precise timing of the additional DNA replication is not a trivial issue. Uzzell and Goldblatt (1967) proposed that premeiotic doubling may have evolved as an escape from the presumed synaptic difficulties in an allodiploid but Cuellar (1974) suggested that, premeiotic restitution cannot be directly selected for by aberrations occurring at a later stage. However, if as Session's (1982) suggests, the reduplication occurs after the initial period of synapsis, then a more realistic selective process can be envisaged. Even so, there are two remaining difficulties with this proposition. First, it invokes high levels of asynapsis. This may be valid in an allotriploid but it

may not be characteristic of putative allodiploids with very similar karyotypes (Cuellar 1974) such as Lepidodactylus and Lepidophyma unless, of course, the pairing failure is genetic rather than mechanical in origin (section 1.3-iv).

Second, the meiotic mechanism must be transmissible and stable which implies a genetic causation rather than purely mechanical factors (Cuellar loc. cit.). Sessions (1982) proposed that mutations, which occur at a relatively high frequency in many interspecific hybrids (e.g. Woodruff and Thompson 1980; Shaw et al. 1983) would subsequently stabilise the novel meiotic process. Indeed, single gene mutants that result in apomixis or intrameiotic restitution have been identified in a wide variety of organisms although, despite the large number of mutants now known, none appear to cause an additional DNA replication early in meiosis. (Baker et al. 1976; Golubovskaya 1979). The results of hybridisation experiments involving apomictic Poeciliopsis (reviewed in Schultz 1977) indicate that, in this case, the novel meiotic mechanisms is not maintained by mutation. When Poeciliopsis monarcha-lucida, which transmits only the maternal monarcha genome, is backcrossed to extant male monarcha, bisexual reproduction (albeit imperfect; Leslie and Vrijenhoek 1980) is restored. This observation suggests that the altered meiotic process may be perpetuated by a continuing and highly specific imbalance in the genetic control of the early stages of meiosis rather than by new mutations.

Unfortunately, the genetic basis of the control of meiotic DNA replication is poorly understood. However, the studies of oogenesis in the triclad planarian Dugesia (reviewed in Benazzi-Lentati 1970) show that the control is complex and multigenic. Dugesia is a

hermaphroditic genus that exists as three biotypes that differ in the mode of oogenesis employed. These are, a diploid and bisexual biotype and two gynogenetic forms, one of which is an apomictic polyploid and the other, an automictic polyploid with premeiotic doubling. Extensive studies of gametogenesis in hybrids between these biotypes showed that the different oogenetic phenotypes were under separate genetic control.

A second important conclusion of these studies was that spermatogenesis and oogenesis are under the control of distinct genetic or epigenetic systems since spermatogenesis in each of the biotypes with altered oogenesis was subject to distinctive meiotic abnormalities. This conclusion is substantiated by the absence of meiotic reduplication in rare males of Lacerta (Darevsky and Kupriyanova 1982) and several invertebrates in which females are automictic with pachytene reduplication (Pijnacker and Koch 1975; Pijnacker and Ferwerda 1978).

In concert, these data suggest that the induction of meiotic reduplication is most probably the result of complex genetic interactions rather than single gene mutations as suggested by Cuellar (1974) and assumed in theoretical models that contrast the consequences of sexuality with subsexuality (section 3.1). The alternative "genetic-balance" hypothesis, alluded to by Bogart (1980) and Turner (1982), is compatible with the complex genetic control of meiosis. In its simplest form, the model suggests that meiotic reduplication is a result of a perpetual imbalance in the genetic control of meiosis.

Recent studies on the meiosis of interspecific hybrids between some bisexual taxa substantiate this hypothesis. In crosses between species of Xenopus (Muller 1977) and Caledia respectively (Shaw and

Wilkinson 1978) high levels of meiotic reduplication leading to the production of diploid gametes were observed. Moreover, in both cases some F1 hybrids proved capable of subsexual reproduction (Kobel and DuPasquier 1975; Bogart 1980; Shaw and Wilkinson loc. cit.) and in the case of Xenopus, the meiotic reduplication was maintained in subsequent generations of triploid backcrosses (Muller 1977). These experiments also showed that crosses between more closely related taxa of Xenopus (Muller 1977) and Caledia (Moran 1981) fail to produce increased ploidy at meiosis which implies that some minimal level of genetic divergence between the parental taxa is required. These observation indicate that meiotic reduplication can be considered as a perturbation of the genetic control of early meiotic events induced directly by hybridisation. The presence of two distinct genomes may therefore enhance the probability of meiotic reduplication occurring in an organism which has the potential for parthenogenetic reproduction. This hypothesis does not deny that polyploid gametes can occur spontaneously in bisexual populations, as it undoubtedly does in many grasshoppers (John and Lewis 1968) and possibly in the Kiribati population of Hemidactylus frenatus (section 3.2.4), but it does suggest that this is far less effective in leading to natural parthenogenesis.

Contrary to the view held by Cuellar (1974) and generally assumed in the hybrid origin hypothesis (e.g. Cole 1975) the taxa involved in the hybridisation events leading to subsexuality need not be separate taxonomic species. This is most clearly demonstrated by Heteronotia (section 3.2.1) and Nactus (3.2.2) and may also apply to Cnemidophorus lemniscatus. The latter species has been widely cited as an exception to the hybrid origin hypothesis since it does not

coexist with other species of Cnemidophorus (Cuellar 1974; Cole 1975; Peccinini-Seale 1981; Bell 1982). However, cytogenetic investigations of the bisexual and parthenogenetic populations of this species (Peccinini-Seale and Frota-Pessoa 1974), suggest that hybridisation between the cytogenetically distinct bisexual populations of C. lemniscatus led to the evolution of the parthenogenetic biotype (Wright cited in Peccinini-Seale and Frota Pessoa loc. cit, White 1978a). An added complication is that preliminary studies of oogenesis suggest that meiotic reduplication may not take place in this species (Peccinini-Seale 1981).

In addition to the absence of a correlation with morphological divergence, it is clear that the induction of subsexuality by hybridisation is independant of the extent of cytogenetic differentiation (cf. Heteronotia and Nactus) and genic divergence (cf. Menidia; Echelle et al. 1983 and Cnemidophorus tesselatus; Parker and Selander 1976) between the hybridising bisexual taxa. If, as suggested by the genetic balance hypothesis, it is only the extent of differentiation between the genes that regulate meiosis that is critical to the establishment of the novel meiotic mechanism, then the divergence in morphology, karyotype and those structural genes assayed by electrophoresis is irrelevant to the issue.

The specific disruption of the genetic control of meiosis that is postulated to lead to the establishment of stable meiotic reduplication would only occur in a minority of hybrids (Neaves 1971; White et al. 1977; Turner 1982). Thus, the frequent failure to induce subsexual reproduction in hybrids between extant populations of the putative parental taxa (e.g. Poecilia; Turner 1982) may simply reflect continued evolution in the bisexual taxa such that the precise genetic

differences that were present at the time of the origin of subsexuality, no longer exist. It does not, as Cuellar (1974) believes, offer compelling evidence against the hybrid origin theory.

In this respect, the experimental hybridisation studies in the genus Warramaba are of particular relevance. This various cytogenetic clones of W. virgo form two groups of separate origin; (i) the widespread standard phylad which arose through hybridisation between northern P196 and P169 Warramaba and (ii) the geographically restricted Boulder-Zanthus clones which had a more recent origin and are derived from hybridisation between southern populations of the bisexual species (reviewed in White and Contreras 1981). Attempts to resynthesise the older standard phylad were without success whereas cross between the southern P169 and P196 populations produced a few synthetic Boulder-Zanthus W. virgo which subsequently proved capable of parthenogenetic reproduction (White et al. 1977; White and Contreras 1978). The extraordinary variation in the results of hybridisation between Poeciliopsis monarcha-latidens and P. latidens (reviewed in Schultz 1977) may also be partly attributable to the requirement for a highly specific divergence of genes controlling meiosis, although the production of all male progeny by some F1 hybrids also indicates a disturbance in the sex determination mechanism (Turner 1982).

Artificial hybridisation experiments also reveal another complication to the induction of subsexual reproduction; it is assymetric. In Caledia (Shaw and Wilkinson 1978), Warramaba (White et al. 1977), Poeciliopsis (Schultz 1977) and most Xenopus combinations (Muller 1977) meiotic reduplication was observed in only one direction of the crosses. The reciprocal combinations were

generally sterile. This directional effect is also evident in natural parthenogens. The analysis of mitochondrial DNA restriction patterns in parthenogenetic Cnemidophorus have demonstrated that the maternal parent of the clones of both C. neomexicanus and C. tesselatus is invariably C. tigris (Brown and Wright 1979; Brown pers. comm.). The reason for this asymmetry is not clear although Turner (1982) suggested that it may be a consequence of interaction between the nucleus and cytoplasm of the oocyte which accords with the partial control of oogenesis by the maternal cytoplasm demonstrated in planarians (Benazzi-Lentati 1970).

Once the meiotic mechanisms required for subsexual reproduction have evolved, the next major constraint is ecological. Organisms with sperm-dependant modes of subsexual reproduction must continue to attract bisexual males in order to produce progeny. However, since the sperm are not transmitted to the subsequent generations by the F1, discrimination between bisexual and hybridogenetic or gynogenetic females by males will be presumably be favoured by selection. This process has been studied in detail in the Poeciliopsis (reviewed in Moore 1975; Schultz 1977). In natural populations sperm appears to be a limiting resource and the hybridogenetic females compete for sperm less effectively than the sympatric bisexual females. Behavioural studies indicate that subordinate males may effect most of the mating with the hybridogenetic females and the sperm-dependance appears to result in frequency - dependant regulation of the number of hybridogenetic females in the population (Moore 1976).

Newly evolved parthenogenetic lineages face an opposing ecological constraint; these females must minimise insemination by sympatric males to avoid clonal extinction through the excessive

production of higher - ploid sterile progeny (Cole 1975; Cuellar 1977). The extent to which this process can cause extinction would be determined by (i) the effectiveness and frequency of insemination and (ii) the degree of decreased fitness of the ensuing progeny.

It is clear that the adoption of parthenogenetic reproduction per se does not lead to premating isolation. Males of Cnemidophorus do not appear to discriminate between bisexual and parthenogenetic females (Neaves 1971; Cuellar and McKinney 1976; Cole 1979) and in areas of sympatry between bisexual and subsexual Lacerta, sterile triploids produced by backcrossing may constitute up to 10% of the population (Uzzell and Darevsky 1975). In fact, the production of sterile backcross hybrids has been suggested as one causal factor for the rarity of sympatry between parthenogenetic populations and their bisexual progenitors (Uzzell and Daversky 1975; Cuellar 1977; White and Contreras 1979). However, it should be noted that the insemination of parthenogenetic females need not lead to fertilisation. In both Lacerta (Darevsky and Danielyan 1968) and Cnemidophorus (Cole 1979) the majority of progeny from such matings are parthenogenetically produced which led Darevsky and Danielyan (loc. cit.) to suggest that the cell membrane of the oocyte presented at least a partial barrier to fertilisation. Although the existance of premating isolation has not been investigated in H. binoei, the rarity of tetraploids, despite the extremely widespread sympatry between the bisexual diploids and parthenogenetic triploids (see Fig. 3.22) may be attributable to such a fertilisation barrier.

The restriction of subsexual vertebrates to either diploidy or triploidy (Table 3.11) is enigmatic. White (1973) suggested four potential causes of the restriction of ploidy levels in subsexual

vertebrates; (i) the absence of closely related bisexual taxa, (ii) cytomechanical restrictions, (iii) the absence of adaptive superiority of polyploids and (iv) a disturbance of the meiotic mechanisms responsible for parthenogenesis. The first of these is unlikely to apply to subsexual organisms of hybrid origin since repetitive backcrossing at the time of their origin should result in ploidy levels greater than triploidy if no other restrictions are operative. As far as the cytomechanical restriction is concerned, a limit to the number of bivalents that can be accommodated on the spindle, and still allow for regular disjunction at anaphase, may be particular relevance to organisms with meiotic reduplication. However the occurrence of decaploid weevils (Takenonchi 1983) and dodeca ploid earthworms (Muldal 1952) show that this restriction is not general.

The relative fitness of allodiploids and allotriploids may be important in subsexual vertebrates. In interspecific hybrids of Rana (Bogart 1980; Elinson and Briedis 1981) and intergeneric hybrids of some fish (Beck and Biggers 1982) the viability of the allodiploids is significantly lower than in the allotriploids produced by backcrossing the allodiploid to one of the parental taxa. Clearly, in some hybrids the poor coadaptation of the divergent genomes can be circumvented through the addition of another chromosome set from one of the parental species and this factor may contribute to the exclusive triploidy in some parthenogenetic lineages (e.g. Heteronotia). In such cases, the subsequent evolution of a tetraploid with two genomes of each parental species (i.e. AABB) may lead to the same reduced viability as was the case in the initial allodiploid (AB). In fact, it is quite remarkable that although numerious viable backcross tetraploids have been identified in vertebrates (e.g. Neaves 1971; Lowe et al.

1970a; Cole 1979, and see section 3.2.1. - vi) none are of the AABB type. However, this explanation fails to account for the evolutionary limitations on other possible genomic combinations in tetraploids (e.g. AAAB) which are viable but apparently sterile.

In this respect the genetic-balance hypothesis for the control of meiotic reduplication is relevant and pertains to White's (1973) fourth factor; the disturbance of the novel meiotic mechanism by the addition of further genomes. This process can be envisaged to act in two distinct ways and to result in the observed ploidy distribution found in subsexual vertebrates. On the one hand, the genetic balance in the initial allodiploid may result in imperfect control of the meiotic reduplication process if the divergence between the genes regulating oogenesis is too great. The addition of another genome from either of the parental taxa could then stabilise the meiotic process and therefore increase the fitness of the allotriploid relative to the allodiploid. There is no direct evidence for this proposition. While the meiotic reduplication is maintained in triploid backcrosses of Xenopus (Muller 1977) and Warramaba (White et al. 1977) there is no comparative data on the long term relative stability of the meiotic cycle for the two ploidy levels of these species. The absence of the postulated allodiploids in parthenogenetic organisms such as Heteronotia suggests that the postulated allodiploid form was ephemeral, presumably as a result of poor viability (see above) or imperfectly regulated meiosis.

On the other hand, the addition of an extra genome may disrupt the genetic balance of an allodiploid lineage with stabilised meiotic reduplication to the extent that it results in sterility (e.g. Lacerta; Darevsky 1966; Cnemidophorus; Cuellar and McKinney 1976) or a

novel meiotic mechanism as appears to be the case in Poeciliopsis (Table 3.11; Schultz 1977). There are only two parthenogenetic vertebrates that are known to exist as both diploid and triploid parthenotes; Cnemidophorus tesselatus and Lepidodactylus lugubris. In the former species, the triploid clones appear to be more geographically restricted, (Zweifel 1965), have lower clonal diversity (Parker and Selander, 1976) and, on the basis of preliminary data, have lower fecundity (Maslin 1971). The reduced fecundity, may be a consequence of meiotic irregularities and this possibility appears worthy of further study. There is no comparative data on the relative fecundity of the diploid and triploid L. lugubris and, once again, this requires investigation.

The genetic balance hypothesis can be extended to still further increases in ploidy. Thus backcrossing of triploid lineages with a stable meiotic mechanism to bisexual populations could be expected to interfere with the regulation of the novel meiotic process. As a result, tetraploids would be sterile, which is generally the case (Lowe et al. 1970a; Cuellar and McKinney 1976). However, note that not all such tetraploids are incapable of producing eggs. For example, Neaves (1971) reported the production of eggs by a tetraploid backcross between Cnemidophorus exanguis and C. inornatus but unfortunately the viability of these eggs was not ascertained. The observations in Heteronotia are of particular relevance here. A backcross tetraploid of one type (clone A-1a x bisexual CA6) had non functional ovaries whereas another genetically distinct tetraploid (clone A-1c x EA6) had normal ovaries; corpora lutea indicating the production of eggs. Thus it appears that the genetic constitution of a tetraploid may affect its ability to at least proceed through oogenesis.

Oogenesis has not been directly observed in any backcross tetraploid reptiles and such a study is clearly needed. If stable meiotic restitution was maintained in these tetraploids then it would offer strong evidence against the genetic-balance hypothesis. Alternatively, the production of diploid ova could provide a step towards the establishment of a bisexual allotetraploid lineage in the manner described by Astaurov (1969). However, as Bogart (1980) emphasised, the rarity of tetraploids makes it more probable that diploid ova would be fertilised by haploid sperm (from the sympatric bisexual diploid population) resulting in triploid progeny, which may or may not regain the capacity for parthenogenetic reproduction.

The genetic-balance hypothesis described above is obviously speculative given the paucity of detailed meiotic studies on parthenogenetic vertebrates and the fragmentary knowledge of the genetic and cytoplasmic control of oogenesis. Nevertheless, the hypothesis is amenable to experimental study. In addition to extending the studies of hybridisation and oogenesis in taxa such as Caledia and Xenopus, the potential for nuclear transplantation studies in the latter organism offer a valuable opportunity to analyse the nature of the apparent maternal cytoplasmic effects on the control of oogenesis.

The elucidation of the evolutionary constraints that operate on the secondary origin of subsexual reproduction in vertebrates is obviously critical to the evaluation of the historical hypothesis for the predominance of sexual reproduction in vertebrates. The above development of the genetic-balance hypothesis has repeatedly stressed the highly specific requirements necessary if hybridisation is to result

in the required patterns of oogenesis. With specific reference to the evolution of automixis (with intra- or post-meiotic restitution) via a tychoparthenogenetic stage, Templeton (1982b) identified two selective thresholds that will be encountered and these are also of particular relevance to a hybrid origin. In the first phase there is strong selection for coadaptation between the two genomes of an allodiploid insofar as it effects their relative viability. In the case of an allodiploid, this process is expected to eliminate all but a few hybrid combinations even if the majority are capable of meiotic reduplication (Turner 1982; White and Contreras 1978). The second phase concerns selection for the efficiency of parthenogenetic reproduction. The imperfections of the meiotic process evident in the Rana esculenta hybridogenetic system (Uzzell et al. 1977) and Ambystoma platineum (Sessions 1982) together with the variation in fecundity between the hemiclones of Poeciliopsis (Schultz 1982) demonstrates that optimal reproductive efficiency is not achieved immediately as the result of some hybrid origins. By contrast, some other subsexual vertebrates have achieved a reproductive capacity equivalent to (Schall 1978), or possibly greater than (Darevsky 1966), their bisexual female counterparts.

As a result of these restrictions, the origin of stable and effective parthenogenetic reproduction in vertebrates is likely to be a very rare event. This substantiates William's (1975) claim that the predominance of sexual reproduction in low fecundity organisms is at least partly a result of historical restrictions on the secondary evolution of alternative reproductive mechanisms. In other words, the genetic complexity of the control of meiosis imposes evolutionary inertia on the mode of reproduction.

Bell (1982) arrived at two predictions from this "historical" hypothesis of which only one is relevant to the origin of parthenogenesis through hybridisation (see section 3.1 - i); the correlates of parthenogenesis should be taxonomic rather than ecological. In terms of its application to vertebrates, this prediction is based on the rather speculative premise that certain taxonomic groups are predisposed towards alterations in the genetic control of meiosis and subsequent hybridisation. With this assumption in mind, it is clear that the taxonomic distribution of subsexuality in vertebrates is markedly non-random (Tables 3.12 and 3.13) and so provides further support for the historical hypothesis. Nevertheless, even if the conditions for the origin of subsexuality are as restrictive as suggested aobve, the subsequent evolutionary and ecological constraints on established subsexual lineages of vertebrates remain an important consideration which will be the subject of the next two sections.

3.3.2. The Genetic Diversity of Subsexual Vertebrates

Two distinct forms of genetic variation, which differ in their evolutionary consequences, must be recognised within subsexual taxa. Extensive intra-clonal heterozygosity may be the consequence of a hybrid origin between genetically distinct bisexual taxa (White 1970) whereas interclonal diversity may be a result of recurrent hybridisation (e.g. Vrijenhoek 1979) or mutation within a subsexual lineage (e.g. Suomailainen et al. 1976). Clearly only the latter process is of significance to the long term evolutionary potential of subsexual biotypes and for the extent of mutation to be quantified, detailed and extensive comparative studies of these taxa and their

presumed ancestors are required (see section 3.1). Some of the clearest evidence for mutation subsequent to a hybrid origin concerns chromosome rearrangements and these data will be the subject of the final section of this chapter.

As predicted by the hybrid origin model, the heterozygosity of some subsexual vertebrates is extremely high. In Cnemidophorus tesselatus the diploid and triploid clones have heterozygosities of .56 and .71 respectively (Parker and Selander 1976) although some other subsexual taxa of hybrid origin have substantially lower heterozygosity (e.g. Menidia, $\bar{H}$ = 0.18; Echelle et al. 1983). The high heterozygosity of subsexual lineages has been claimed to maximise heterosis without genetic load (White 1970) or to provide general purpose genotypes endowed with considerable ecological flexibility (Maslin 1971; Parker 1979b). For example Schultz (1977, 1982) proposed that heterosis may be important in determining the niche breadth and competitive ability of hybrodogenetic Poeciliopsis although the evidence for this is equivocal (Moore 1976; Bulger and Schultz 1982).

The origin of interclonal diversity is frequently obscure. For example, Suomalainen et al. (1976) have proposed that the extensive clonal diversity of curculionid weevils is the result of mutation subsequent to the origin of parthenogenesis although other authors consider that it may be the result of polyphyletic origins, perhaps through hybridisation (White 1978a; Vespalainen and Jarvinen 1979). In the cases of Poeciliopsis (Schultz 1977; Vrijenhoek 1979) and Heteronotia (section 3.2.1) it is clear that the primary cause of clonal diversity is repetitive hybrid origins although in the latter species some of the cytogenetic heteromorphisms are attributable to mutation.

A detailed study of electrophoretic variation in Cnemidophorus tesselatus (Parker and Selander 1976) resolved 12 clones amongst the diploid populations and of these, three appear to be the result of mutation subsequent to the establishment of parthenogenesis.

Thus, it is clear that multiple hybridisation events, backcrossing and mutation may each contribute to the total clonal diversity found within subsexual vertebrates. As a direct consequence of these factors, most adequately sampled taxa reveal polyclonal population structures (Parker 1979a, Bell 1982) which provides the genotypic diversity necessary for the operation of inter-clonal selection (see below).

Comparisons of morphological variation in parthenogenetic vertebrates and their bisexual ancestors have consistently shown that the total phenotypic variation of the former is no less than for the bisexual species (Zweifel 1965; Parker 1979b) as is also the case for an analogous comparison of parthenogenetic and bisexual Warramaba (Atchley 1977a). However within clones (or single population samples) the phenotypic variance is lower than in bisexual populations (Zweifel 1965; Darevsky 1966; Parker 1979b). Those characters in which this pattern is not apparent, may simply have low genetic variance or may be determined by complex genotype environment interactions (Parker 1979b: Atchley 1977b). The high levels of morphological plasticity in some subsexual taxa, analogous to agamic complexes in plants (Grant 1981), was considered by White (1973) to be primarily attributable to polyphyletic origins of subsexuality rather than evolutionary diversification within a lineage.

Although much of the clonal diversity evident in subsexual vertebrates does appear to represent a conservation of different

hybrid genotypes associated with polyphyletic origins, it is clear that this variation presents a substantial basis for differential selection to act between clones. It most definitely does not support the predictions of various models for the maintenance of sexual reproduction that invoke short term selection against subsexual organisms due to their presumed loss of genetic variation (the best man and differential extinction hypotheses, table 3.2).

3.3.3. Environmental Correlates of Subsexuality in Vertebrates

This subject has been recently reviewed by Cuellar (1977) and Bell (1982). Their analyses revealed a remarkably consistent association between subsexual reproduction and unpredictable habitats which is contrary to previous theoretical assertions that subsexual taxa are incapable of adaptation to physically harsh or fluctuating environments (see section 3.1, table 3.2). This sort of geographic pattern is epitomised by the triploid clones of Heteronotia which predominate in the extremely arid and unpredictable central and western deserts of Australia (section 3.2.1-v). This association is certainly not universal since all other known subsexual gekkonids are widely distributed on tropical islands which may be a reflection of their enhanced colonising ability (Cuellar 1977).

The biotic component of environmental variation may be more significant in determining the survival of subsexual lineages (Bell 1982). Two divergent schools of thought have developed concerning the competitive abilities of subsexual vertebrates. It has been proposed that such taxa will have inherently poor competitive abilities or relatively restricted niche requirements compared to their bisexual progenitors (Cueller 1977; White 1978a). This view also constitutes

a basic premise of the differential - extinction and tangled bank models for the maintenance of sex (section 3.1 Table 3.2) and has been invoked to explain the apparent tendancy of parthenogenetic taxa to occupy areas where the bisexual taxa are rare (Wright and Lowe 1968).

Alternatively, consideration of the consequences of clonal competition suggests that a subsexual lineage may eventually comprise several specifically adapted successful clones or fewer general purpose genotypes of broad ecological tolerance (Parker 1979a,b, Vrijenhoek 1979). This process would be facilitated by either the generation of clonal diversity through polyphyletic origins (Parker 1979a) or temporal environmental heterogeneity (Parker et al. 1977; Jaenike and Selander 1979). A closely related proposition is that the fact of hybridity itself may produce greater ecological flexibility than was present in either of the parental bisexual species (Maslin 1971; Schultz 1977, 1982).

Unfortunately there are few precise studies of comparative niche breadth in related bisexual and subsexual vertebrates than can be used to resolve this issue. The most comprehensive analysis concerns the ecological relationships of hybridogenetic and gynogenetic Poeciliopsis with their bisexual host species (reviewed in Schultz 1977, 1982; Vrijenhoek 1979). These data show that the hybridogens have broader thermal tolerances than their related bisexual species and, at least in one case, also have a superior growth rate and attain a larger adult size. Analysis of the trophic niche of gynogenetic Poeciliopsis and their bisexual hosts demonstrated that the latter had more diverse dietary requirements than any single clone, although individual clones occupied different niches and the gynogenetic form

as a whole occupied a wide range of habitats (Vrijenhoek 1978, 1979). Another observation to emerge from these studies is that the density and total niche breadth of Poeciliopsis is proportional to clonal diversity (Schultz 1977; Vrijenhoek 1979) which is consistent with the tangled bank and differential extinction hypotheses (Table 3.2).

The requirement for well developed competitive abilities in these sperm-dependant taxa is probably greater than for parthenogenetic taxa that are capable of colonising areas devoid of bisexual populations (Vrijenhoek 1979). In fact the parthenogenetic populations of vertebrates are commonly ecologically separated from their bisexual relatives. The parthenogenetic species of Lacerta are more arid adapted than their related bisexual species which is reflected by their dominance in drier habitats (Uzzell and Darevsky 1975). The competitive interactions between geographically proximal bisexual and parthenogenetic Cnemidophorus appear to be complex but are poorly understood (Cuellar 1979). In the most detailed study date, Mitchell (1979) found no differences in niche breadth between two bisexual and two parthenogenetic species where they occurred in sympatry. Both Mitchell (loc. cit.) and Cuellar (loc. cit.) suggest that the species of Cnemidophorus are primarily separated on macrohabitat requirements. Finally, Pianka and Pianka (1976) showed that triploid Heteronotia can have an exceptionally broad niche although comparative data for bisexual populations from the same areas is lacking. These limited data on the ecology of parthenogenetic vertebrates, together with their generally polyclonal population structure (section 3.3.2) do not suggest that individual clones necessarily have narrow ecological niches or suffer regular extinction due to their purportedly precise ecological requirements as suggested by Williams (1975).

The effects of broader biotic interactions, such as predator-prey or host-pathogen coevolution, on subsexual populations is a subject of almost total ignorance. Jaenike and Selander (1979) suggested that parasitism may exclude parthenogenetic earthworms from the temporally stable deep-soil habitat but did not provide the critical date to substantiate this claim. No studies have been directed towards comparative analysis of the frequency of parasites and pathogens and their virulence in subsexual and bisexual taxa of vertebrates. Thus, while the Red Queen hypothesis may in theory be a potent force in the maintenance of sexual reproduction (section 3.1), empirical tests of this theory are totally lacking.

3.3.4 Conclusions

Of the various explanations put forward to account for the predominance of sexual reproduction in animals (section 3.1), the historical hypothesis may be the most relevant to vertebrates. According to the genetic-balance hypothesis, developed in section 3.3.1, the conditions for the establishment of a stabilised meiotic mechanism that allows for subsexual reproduction would be highly restrictive. The evolutionary constraints that operate on the few lineages that do become established are not clear. The zoogeographic distributions of these taxa and their substantial clonal diversity appears to negate the predictions of models that invoke rapid clonal extinction in fluctuating environments or a general incapacity of subsexual forms to respond to directional selection (i.e. the Hitch-Hiker, Fisher-Muller, Differential Extinction and Best Man models; Table 3.2). The tangled bank model (Table 3.2) may be applicable to sperm-dependant unisexual vertebrates such as

Poeciliopsis that, to some extent, have ecologically specialised clones. However, the available data for clones of parthenogenetic vertebrates do not appear to conform to this model.

One theory had has not been addressed so far in this discussion is Mullers Ratchet which proposes that deleterious mutations will accumulate in subsexual lineages to the point where they cause clonal extinction (section 3.1). The expected mutations leading to non-functional gene products have in fact been demonstrated to exist in hybridogenetic Poeciliopsis (Leslie and Vrijenhoek 1980) and may also be responsible for the inviability of gynogenetic Rana in which the usual expression of a paternal genome (by hybridogenesis) masks the presence of recessive lethal mutants (Binkert et al. 1983; Graf and Muller 1979). Consequently the accumulation of non-functional mutants may, in the long term, cause the demise of subsexual clones although this process is expected to be delayed in polyploid taxa (Lokki 1976b). Turner (1982) has suggested a positive aspect of non-functional mutants in allodiploids; if these initially suffer from poor coadaptation between their genomes, then the gradual silencing of discordant alleles by mutation may enhance the long term survival of such clones.

The conclusions presented above must be considered speculative given the fragmentary knowledge of the genetics and ecology of subsexual, and particularly parthenogenetic, vertebrates. The primary aim of this discussion has not been to provide dogmatic assertions on the mechanisms which maintain sexual reproduction in vertebrates. Rather, the intention was to review the available data for gekkonids, and vertebrates in general, in the context of the predictions provided by Bell (1982) and to indicate which areas of

future research are of primary importance. As has been previously stressed (White 1978a; Bell 1982), the genetic control of the meiotic mechanisms responsible for parthenogenesis, together with appropriate experimental studies are urgently required. The almost total lack of data regarding competitive interactions between sympatric bisexual and subsexual populations also requires rectification. Further, the basic data required to evaluate the Red Queen hypothesis is not available for vertebrates. In respect of these hypotheses based on biotic interactions, Heteronotia appears to offer excellent material for comparative studies. This species appears to have an unusually broad zone of overlap between the diploid bisexual and triploid subsexual populations (Fig. 3.22) and occurs at sufficient densities to allow for comparative ecological studies and an analysis of pathogen loads.

3.4 Implications for Theories of Chromosome Evolution

It is a widely held view that the extent of chromosomal diversity in bisexual populations is, to a large extent, restricted by purely mechanical meiotic constraints that operate on individuals that are heterozygous for chromosome rearrangements (see section 1.3). In the virtual absence of recombination, automictic parthenogens should be released from such meiotic constraints (White 1975). In fact, some authors disclaim extensive chromosomal heterozygosity as evidence for a hybrid origin of subsexuality on the basis that this variation simply reflects the rapid accumulaton of chromosome rearrangements in the absence of the presumed meiotic barriers (Maslin 1971; Cuellar 1974).

Lokki and Saura (1980) suggested that the phenotypic effects of a loss or duplication of chromosome segments may be masked in

polyploids if two functional genomes remain. This means that, if the circumvention of meiotic constraints is critical, then both diploid and triploid parthenogenetic lineages of reasonable antiquity should have greater cytogenetic divesity than their bisexual counterparts. However, if the second effect, which is reliant on polyploidy, operates alone, then only triploid parthenogens should have increased chromosomal heterozygosity.

Geographically comprehensive studies of chromosome variation in subsexual vertebrates have only been reported for Lepidodactylus lugubris (section 3.2.3), Heteronotia (section 3.2.1) and some species of Cnemidophorus (Table 3.12). The diploid and triploid clones of L. lugubris are characterised by their cytogenetic constancy although the report of 2n = 3x-3 = 63 chromosomes in a population from Japan (Makino and Momma 1949), if accurate, indicates some numerical variation among the triploids.

Within Cnemidophorus, Lowe et al. (1970a,b) alluded to frequent minor cytogenetic variants in both diploid and triploid parthenogenetic species but the details were not presented. However, there are several clear examples of cytogenetic diversification in these parthenogens including centric fusions and the loss or gain of microchromosomes (Table 3.13). Unfortunately, with the possible exception of C. lemniscatus (Peccinini-Seale and Frota Pessoa 1974) there have been no extensive and systematic cytogenetic studies of Cnemidophorus that can be used to compare the extent of cytogenetic diversity in parthenogenetic lineages and their progenitor bisexual species.

In this respect, the cytogenetic study of Heteronotia (section 3.2.1) is unique for vertebrates and parallels the detailed analysis of

cytogenetic variation in Warramaba conducted by White and his colleagues (Webb et al. 1978; White and Contreras 1982). Amongst the triploid Heteronotia only five of the 17 cytogenetically distinct clones so far identified present heteromorphisms for chromosome mutations not attributable to polyphyletic hybrid origins. Further, only two of these, representing three of the 189 triploid individuals analysed, incorporated large structural chromosome rearrangements, namely Robertsonian fusions, that could conceivably be detrimental in a bisexual species. One possible explanation for the restricted chromosomal diversity of the triploid H. binoei is that parthenogenesis has only recently evolved in this species. However this is at variance with the extremely widespread and complex geographic distribution of some of the clones.

In the detailed study of Warramaba virgo and related bisexual species, Webb et al. (1978) and White and Contreras (1982) also found an unexpectedly low level of novel cytogenetic variants which led these authors to suggest that the rearrangements that did occur were being eliminated as a result of deleterious position effects. As is the case for Heteronotia, the clones of W. virgo that carried novel fusions were geographically restricted implying that their fitness was, for some reason, reduced (White and Contreras 1982). Thus both the parthenogenetic vertebrates that have been adequately studied and W. virgo fail to present any evidence for a rapid accumulation of chromosomal rearrangements which are expected in the absence of the presumed mechanical meiotic contraints.

The cytogenetic studies of Heteronotia also provides evidence pertaining to the process of karyotypic orthoselection (White 1975). Bisexual populations of H. binoei exemplify this principle in that the

only extant structural rearrangements of chromosomes are inversions. One of the potential causes of this process is differential mutation wherein certain taxonomic groups are predisposed to a specific class of mutations and not others (White 1975; Peters 1982). In Heteronotia, the regularity of structural rearrangements in the bisexual taxa cannot be a consequence of differential mutation since in the triploids, only one derived clone is the result of an inversion, whereas two result from Robertsonian fusions.